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ANATOMICAL FINDINGS IN A CASE OF FACIAL PARALYSIS OF TEN DAYS' DURATION IN A GENERAL PARALYTIC, WITH REMARKS ON THE TERMINATION OF THE 'AUDITORY' NERVES.

BY ADOLF MEYER, M.D., WORCESTER LUNATIC HOSPITAL, WORCESTER, MASS.

PLATES XLIV AND XLV.

In the *Neurologisches Centralblatt* of August, 1896, Dr. E. Flatau described a case of facial paralysis of several months' duration. He found marked alterations in the cells of the nucleus of the same side and plain degeneration of the fibres. Further, with the Marchi stain he observed a decussating part of the root, such as Obersteiner pictures. The earlier descriptions of peripheral facial paralysis include no data on the condition of the medulla.

The following case, of much shorter duration, has been examined with the Nissl method only, but offered several points worth reporting.

The patient, C. C., 42 years old, was admitted November, 1894, with general paralysis, which took the course of the demented form. The patient died in a convulsion on March 26, 1896. Ten days before death a paralysis of the muscles of the left side of the face was noticed. Nothing was found to indicate middle ear disease, and to all appearance the paralysis was like the so-called rheumatic facial palsy. It was impossible on account of the patient's dementia to

examine the hearing or taste. Paralysis was complete. An autopsy was made nineteen hours after death and part of the region of the facial nucleus was fixed with alcohol, 94 per cent. The rest, together with the middle ear, was preserved in 10 per cent formalin.

The examination of the *medulla oblongata* gave the following results: On the right side nothing abnormal was noticed; on the left side the Nissl specimens showed to the naked eye a blue streak along the distribution of the auditory nerve in its ventral and dorsal roots (Plate XLIV, Fig. 1). Microscopically, the termination of these roots proved to be infiltrated with a great number of neuroglia cells. In explanation of Fig. 2 (Plate XLIV) it is worth stating that the neuroglia accumulation is not so marked close to the cell bodies of the so-called auditory nuclei, where we would find it in infiltration due to typhoid fever, and even more in typhus, but rather in the areas of end-arborizations, where we find neuroglia cells in all stages up to the form called "spider cells." The *dorsal nucleus of the eighth nerve* and the *ventral nucleus* were both infiltrated, and between them there was a relatively free place with the large cells of the *nucleus of Deiters*. The cells of the infiltrated portion showed a marked decay of the large granules, and in the most of them the cell body was a little shrunken, and the nucleus was small, showing a tendency to be dislocated towards the surface. The cells of the free *Deiters' nucleus* were perfectly normal, resembling on the whole Nissl's "motor type." The *facial nucleus* showed the characteristic changes described in the experiments of Nissl on the rabbit: swelling of most of the cells, dust-like decay of the granules, in many cells an almost homogeneous swelling of part of the cell body, dislocation of the nucleus to the surface in many of the cells and somewhat irregular contour of the nuclear membrane. In many places the homogeneous swelling seems, however, to go over into the formation of a fine yellowish pigment-like substance (Plate XLV, Fig. 3). The nuclei in most of the cells showed a diffusely bluish color. The nucleoli of many of the cells show little vacuoles and notches (sometimes two) along the margin; occasionally two nucleoli are seen, and quite frequently one, two or even three accessory nucleoli. All the cells in the nucleus are more or less affected; there is only little neuroglia infiltration.

It is worth mentioning that along the course of both the seventh and eighth *nerves* no infiltration is noticed. It is limited altogether to the region of the end-arborizations of the 'auditory' nerve. Marchi and Weigert stains were not used.

The examination of the contents of the internal auditory canal brought out the cause of the condition found. The periosteum of the canal showed a number of hæmorrhages and round cell infiltration, by which both the nerves and the ganglion had been compressed, the ganglion too being strongly invaded by leucocytes. The nerve cells in the hæmatoxylin sections appear granulated, brownish, as if transformed into pigment bags. The nucleus is oval or round, rather small, diffusely and poorly stained, often eccentric (Plate XLV, Fig. 4). In a few of the cells a nucleolus is still visible. The infiltration does not extend along the nerves towards the medulla. It is largely of mononuclear cells in the periosteal tissue; in the ganglion, however, there are many polynuclear cells.

The findings can be summed up as follows: In a case of general paralysis a hæmorrhagic infiltration of the periosteum of the internal auditory canal has led to an invasion and compression of the auditory cells and the nerve, and perhaps later the facial nerve. Ten days after onset of the facial paralysis the nucleus of the facial nerve, of the same side only, showed the typical changes of reaction to a peripheral lesion as described by Nissl and others in experiments on animals. There was no evidence of decussation of elements of the seventh nerve, the cells of the right nucleus being all intact in the numerous sections examined. The terminal nuclei of the eighth, both the dorsal and the ventral, showed well circumscribed neuroglia-cell infiltration, whereas the nucleus of Deiters is almost completely free. The central auditory cells are also slightly affected within the region of infiltration.

The case is of some interest, as it shows plainly two forms of reaction to injury of peripheral nerves, the reaction in the sensory elements being first described here, and found owing to the lesion of the cells of the sensory nerve.

The case speaks against a decussation of the roots of the facial

nerve and for a relative independence of the nucleus of Deiters from the auditory nerve endings (against the views of Bechterew), and gives an illustration of how an affection of one fibre system may involve the cells of another system with which it comes in contact. In many respects the case is as clear as an experiment.

DESCRIPTION OF PLATES XLIV AND XLV.

Plate XLIV.

Fig. 1.—Facial cochlear and vestibular nuclei and Deiters' nucleus. Drawn with Reichert's lens 20 from a specimen stained with Nissl's method. Infiltration of cochlear and vestibular nuclei.

Fig. 2.—A small field from a thin section of the cochlear nucleus; central auditory cells and neuroglia infiltration. The largest spider cells at a distance from the auditory cells. Nissl's stain. Leitz oil-immersion $\frac{1}{12}$, Ocular 2.

Plate XLV.

Fig. 3.—Cells from facial nucleus. Normal side (ganglion cell at left hand of figure) and paralyzed side. Leitz oil-immersion $\frac{1}{12}$.

Fig. 4.—Cell from the infiltrated ganglion in the internal auditory canal and several polynuclear leucocytes. Hæmatoxylin-fuchsin. Leitz oil-immersion $\frac{1}{12}$.

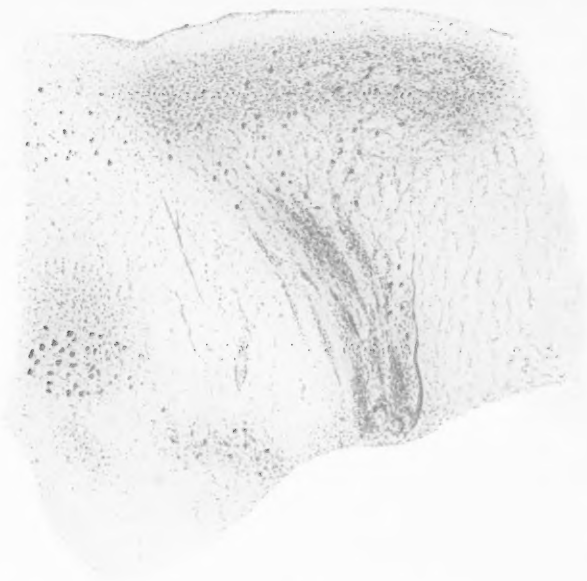


FIG. 1.

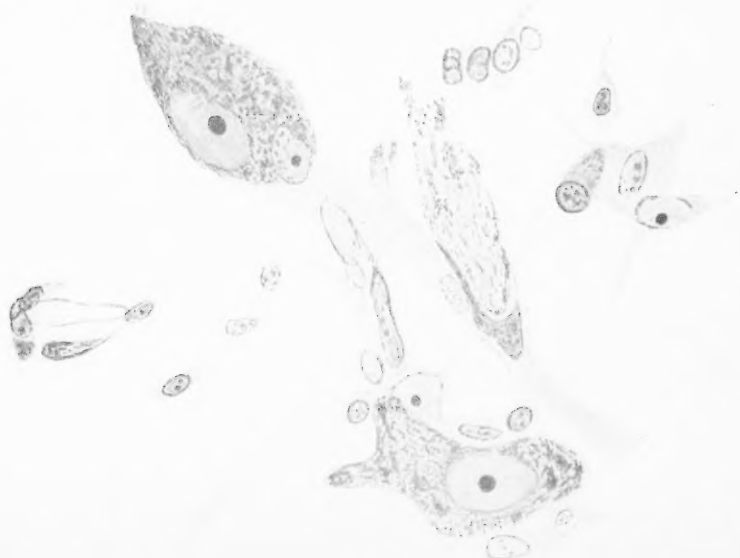


FIG. 2.

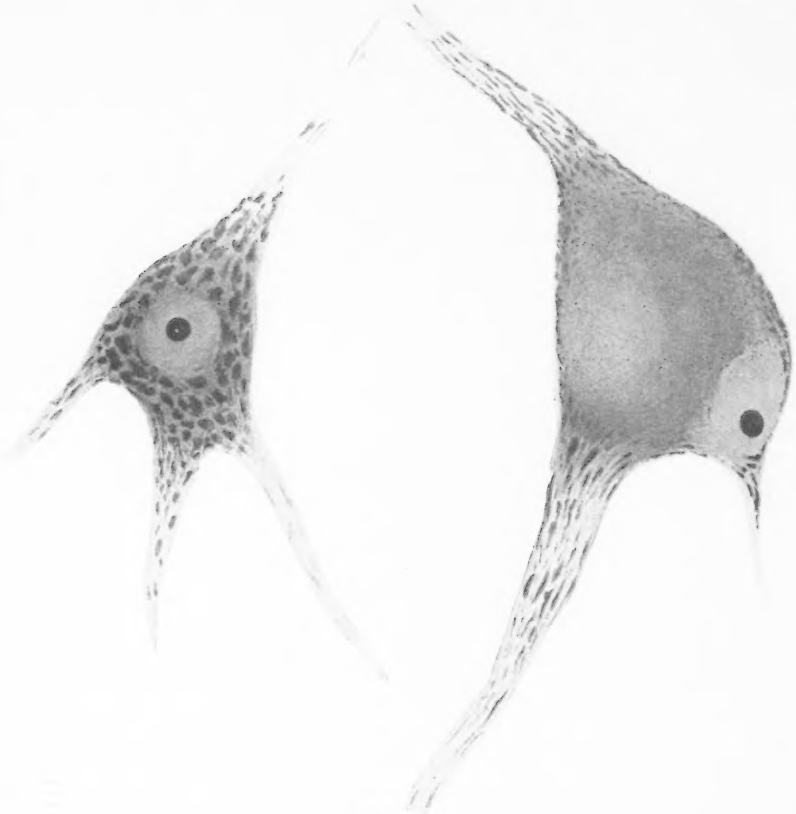


FIG. 3.

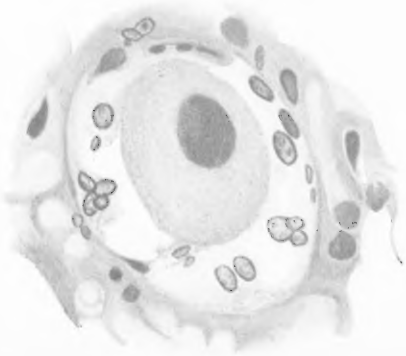


FIG. 4.

A CONTRIBUTION TO THE STUDY OF HUMAN NEUROGLIA.

By Edward Wyllys Taylor, M. D.

(*From the Scars Pathological Laboratory of the Harvard Medical School.*)

Plates XLVI–XLIX.

The neuroglia has been a peculiarly elusive tissue. What knowledge we have had has been gained laboriously and by means of inadequate methods. Certain of the more recent means of staining, while throwing an added light on various histological details, have tended rather to obscure than elucidate the real problem at issue. This is unquestionably true of the numerous silver precipitations which go under the general name of the Golgi method. With all the wealth of knowledge it has given us, there can also be no doubt that from its essential crudeness this method has tended to perpetuate error. It has failed, in the hands even of its most skilful advocates, to demonstrate the intimate histological peculiarities which we need to know and understand. It should, however, at once be said that the function of the method lies in a different sphere, for which it is admirably adapted. But there appears to be a certain danger that it may be misused in the attempted solving of questions for which it is inadequate. One of these deals with the histological structure of neuroglia. A method which colors by precipitation is *a priori* incapable of giving us the information we require; it must, of necessity, be confusing in its pictures of structural detail. Nevertheless, in connection with the neuroglia, conclusions of moment have been drawn from its use* which, in the light of more appropriate methods, must be regarded as misleading, if not actually wrong.

* von Lenhossék, Der feinere Bau des Nervensystems. Berlin, 1895.

With the appearance of Weigert's* long-awaited monograph, and the almost simultaneous publication of Mallory's† analogous method, an absolutely new light has been thrown upon the question. Working independently, the two investigators, using practically an identical method, have reached the same general conclusion of the disassociation of neuroglia cells and neuroglia fibres in the adult human nervous system, a conclusion which has been rendered possible by the use of a differential chemical stain. There can be no doubt of the essential correctness of this histological fact, and it is not our purpose to argue in detail its claim to acceptance. Weigert's monograph, though defective in certain respects, leaves no reasonable doubt in one's mind of the essential justice of his claim, which Mallory shares. It goes without saying that many questions remain unanswered, and that we must reconstruct in great measure our conception of the neuroglia, as with the advent of the neuron we have been faithfully doing in regard to the architecture of the nervous system in general.

Stroebe has vigorously and skilfully opposed Weigert's views in the most significant monograph on the subject of "Glioma" published within the last few years,‡ and also in the discussion of the Naturforscherversammlung, held at Frankfurt in September, 1896, on the structure of newly formed neuroglia in pathological conditions.§ Stroebe bases his claims on results furnished by Mallory's phosphomolybdic-acid-hæmatoxylin stain, a method whose advantages and shortcomings in the solving of the question at issue we shall have occasion to allude to later.

Whatever may be the ultimate outcome of the controversy, it is clearly evident that the significance of the new methods is extremely great. If, as there is every reason to suppose will be the case, they fulfil their early promise, we shall have, what we have so much needed, a definite and certain means of studying normal conditions and

* Weigert, Beiträge zur Kenntnis der normalen menschlichen Neuroglia. Frankfurt a. M., 1895.

† Mallory, *Centralbl. f. allg. Pathol. u. path. Anat.*, vi (1895), 753.

‡ Stroebe, Ueber Entstehung und Bau der Gehirngliome. Ziegler's *Beiträge*, xviii (1895), 405.

§ Stroebe, *Centralbl. f. allg. Path. u. path. Anat.*, vii (1896), 864.

pathological processes, with the accompanying hope that some of the problems of neuroglial proliferation may at least be intelligently met.

It is the purpose of the present paper to bring up certain questions which this most recent work forces upon our attention, in connection with a study, chiefly anatomical, of two brain tumors of peculiar interest.

The clinical history* and gross pathological anatomy of the first and more significant case is as follows:

Case I. Anna H., 8 years, American; admitted to Massachusetts General Hospital, Nov. 29, 1895.

Family history negative. Has had whooping cough, measles, chicken pox, bronchitis. Four years ago patient began to stammer, and developed spasm of the right foot and leg. The motor disturbance apparently improved, but the speech remained hesitating. This condition continued essentially unchanged for two years. At the end of this time, paresis of the right leg and spasm of right arm were noted. Increase of symptoms took place during the following months, so that an attempt to go up stairs became difficult. In June, 1895, vomiting appeared independent of eating, and more frequent at night; in August, marked defect in vision. Appetite and sleep poor. Defective control of bladder and rectum. Increasing difficulty with speech. Some headache.

Physical Examination on entrance to hospital. Patient somewhat emaciated, dull, cries out occasionally. Right arm and leg smaller than left. Paralysis of right facial nerve. Hemiparesis, right arm and leg. Right forearm and fingers flexed. Pupils dilated. Double optic neuritis.

Sleeps much of the time; shows but slight evidence of pain. Incontinence of urine and feces. Moves the right arm at shoulder.

Operation by Dr. S. J. Mixter, December 5. Ether. Usual incision. Dura normal, bulging. Dura incised. Brain bulged through opening. Injection of vessels, otherwise normal appearances. Bone returned; dura loosely sutured. Much shock from operation. Slight improvement under stimulation. Death the following morning.

During the patient's stay in the hospital her temperature was for the most part slightly above normal, not, however, reaching higher than 100.2°F.

Autopsy, by Dr. W. F. Whitney. Skull alone opened. Calvaria normal. Convolutions somewhat flattened. Cortex normal in appearance. Lying beneath the floor of the left ventricle was a cystic cavity,

* Taken from Records, Mass. General Hospital.

containing about two ounces of serous fluid. Into this projected a new growth, apparently arising in and replacing the optic thalamus and corpus striatum. The growth was also detected in the region of the optic chiasm, close to which were several small cystic protuberances.

Further examination of the fresh specimen was deferred for fear of destroying relations in the process of manipulation. The brain was preserved entire in formalin.

After hardening, frontal sections were made, passing respectively through the tumor at its point of greatest development, viz., two centimetres anterior to the pons (see Plate XLVI); through the pulvinaria of the optic thalami, and through the cerebellum and posterior horns of the lateral ventricles.

For the preservation of topographical detail the method of sectioning after formalin hardening is altogether preferable to the immediate study of the fresh specimen. Cross section No. 1 (Plate XLVI), taken through the tips of the temporal lobes and the optic chiasm, 2 cm. anterior to the pons, shows the gross appearances and position of the new growth. At this level it lies as a solid discrete mass about the size of a walnut, chiefly in the left hemisphere, from which it undoubtedly originated, extending, however, considerably over the median line, leading to the distortion of the third ventricle, as shown in the plate. The tumor itself is dense and granular in appearance, suggesting fibrous tissue. Surrounding the main tumor mass on every side are larger and smaller cysts, which were in part filled with a serous fluid and in part with a gelatinous substance. These cysts originally had definite walls, as shown in the partially reconstructed plate, and were entirely unconnected with the ventricular cavities. The cyst of largest size was situated in the left lateral ventricle, filling a large portion of its cavity, and contained a fluid not unlike cerebro-spinal fluid in appearance and consistence. The tumor might be described as extending into this large cyst-cavity, from which it is, however, sharply separated. A similar but much smaller cyst lies at the base of the brain, pressing upon and, no doubt, in large measure destroying the optic nerves in the neighborhood of the chiasm. Smaller cysts of similar character are to be seen, differing from the larger ones chiefly in the fact that their contents are gelatinous.

A section (No. 2) made 2 cm. posterior to that already described, and passing through the posterior portions of the optic thalami, shows the tumor to have reached its limit in this direction. Occupying the pulvinar of the left thalamus is a cyst filled, as were many of the others, with a jelly-like mass. Anteriorly, the growth reaches only slightly beyond the anterior extremity of the nucleus caudatus.

Both lateral ventricles are moderately dilated, the left somewhat more than the right, particularly in its descending horn. The centrum semiovale is symmetrically reduced in size; the corpus callosum is flattened and the fornix difficult to follow in its relation to the corpus callosum. The basal ganglia of the right side have suffered somewhat through pressure. It is altogether probable that the tumor had its origin in the optic thalamus or corpus striatum of the left hemisphere. These ganglia are almost completely replaced by the new growth. Nothing corresponding to the caudate nucleus is to be made out; the putamen of the lenticular nucleus only is visible with the claustrum and external capsule. The optic thalamus is wholly converted into tumor. On the right side the ganglia have suffered only through pressure.

To summarize the macroscopic appearances: We have a dense discrete tumor replacing the basal ganglia of the left hemisphere, associated with extensive cyst formation and consequent distortion both of the cavities and the parenchyma of the brain.*

Methods. After hardening in formalin sections were taken from several portions of the growth which forms the basis of this study, and prepared in various ways for special staining methods. For purposes of comparison two other brain tumors were particularly studied, one a rapidly growing glioma with proliferation of connective tissue—already alluded to—and the other a sarcoma, together with tumors already in my collection. A variety of staining methods has been used, of which the differential neuroglia stain of Mallory† is by far the most satisfactory. Slight modifications of the method as originally published have been made by its author,‡ which justify the following detailed description:

Small pieces of the tissue to be examined, from 2 to 5 mm. in thickness, are hardened in

1. Formol, 10% aqueous solution, 4 or more days.
2. Picric acid (saturated aqueous sol.), 4-5 days.
3. Directly into bichromate of ammonium (5% aq. sol.), 4-5 days.
4. 80% alcohol.
5. 95% alcohol.
6. Imbed in celloidin.
7. Cut, thin sections.

* For a tumor very similar in gross appearance see Oppenheim, Die Geschwülste des Gehirns, in Spec. Pathol. u. Ther., herausg. von H. Nothnagel, ix, I, 3, p. 5. Wien, 1896.

† Loc. cit.

‡ Mallory, *The Journal of Experimental Medicine*, ii, 532.

Staining. 8. Fasten section to slide with ether vapor out of 95% alcohol, and harden in 80% alcohol.

9. Stain with solution of aniline oil-gentian violet, 15-20 minutes.

10. Wash in salt solution.

11. I, K I, ½-1 minute.

12. Wash with water.

13. Dry with filter paper.

14. Aniline oil and xylol, equal parts. Several changes, blotting after each washing, until clearly differentiated.

15. Xylol, wash thoroughly 3-4 times.

16. Canada balsam.

It will be seen that this method is essentially the same in principle as that published by Weigert, with the advantage that it is somewhat less complex and therefore easier of execution. The difference in the two methods lies in great measure in the preliminary hardening and fixing, Weigert using a fixative, followed by a mordant and afterwards by a reducing agent, whereas Mallory employs a mordant after the reducing agent.* The procedure of staining in both cases is a slight modification of Weigert's well known fibrin method. In the Mallory method, however, no intensifier is used, for which Weigert employs chromogen, the danger being that with the use of an intensifying agent structures other than neuroglia may stain, *e. g.*, neuraxon processes.†

In this investigation Mallory's method has been used to the exclusion of Weigert's largely as a matter of convenience, since the two are identical in their results. The value of the method, over others hitherto employed in the study of neuroglia, lies in its delicacy, in its absolutely selective action, and in its property of staining nuclei and neuroglia fibres to the exclusion of the protoplasm of the neuroglia cells. Such a combination of attributes in a single method has not hitherto been attained; its significance to pathological histology can hardly be overestimated. What it has already done for normal histology Weigert's exhaustive monograph has shown.

Other methods used are those already familiar in neuropathological work, with the addition of one recently published by Mallory.

1. Mallory's phospho-molybdic-acid-hæmatoxylin.
2. Van Gieson's hæmatoxylin-picric-acid-fuchsin.

* For details, see the respective publications of Weigert and Mallory, to which the references have already been given.

† Weigert has himself recently stated that in addition to the glia fibres, fibrin and degenerated neuraxon processes may stain. He does not, however, admit that normal neuraxon processes may do so also, which is possible when an intensifying agent is used.

3. Weigert's myeline-sheath-copper-hæmatoxylin method, with the use of the quick hardening solution following formalin (10%).

4. Alum-hæmatoxylin-eosin.

5. Mallory's phospho-tungstic-acid-hæmatoxylin.*

Each of these methods has given information of value, and has likewise demonstrated its shortcomings as applied to the investigation of a tissue to which its use is not essentially adapted.

Histological Examination. Examination with low power shows the mass of the tumor to be made up essentially of finer and coarser neuroglia fibres, irregularly associated in bundles of varying size, giving the general appearance of a dense fibrous tissue (Plate XLVII, Fig. 1). The fibre bundles are in many places seen to be cut transversely or obliquely, and in general do not lie predominantly in any one plane. Throughout the entire tumor spaces are visible between the various fibre bundles, varying in size and everywhere more or less completely filled with a homogeneous material and desquamated cells of somewhat uncertain character and origin. They are probably endothelial and large and small mononuclear lymphoid cells. It is probable that the spaces referred to are lymph channels, whose relation to the vessels is inconstant. Occasionally a capillary may be seen in the middle of such a space, but more often the spaces lie in the substance of the tumor quite free from and independent of contained vessels.

Vessels with thin walls, containing a small amount of blood, are numerous. They apparently bear no distinct histological relation to the surrounding proliferated neuroglia. In the gliomatous tissue proper, comparatively few nuclei are to be made out, when studied with a high power, and such as are present for the most part cannot be shown to bear any clear relationship to the greatly developed fibres (Plate XLVII, Fig. 2). The cellular element is absolutely in the background, the essential and striking feature of the tumor being the presence of fibres, whose number is out of all proportion to that of the cells. Careful study of individual fibres shows them to be coarsely wavy in outline, extremely like those forming a dense, coarse, fibrous tissue, and rarely showing sharp bends or angles in their course. In calibre there is much variation. There are many fine fibres, but nowhere so delicate as seen under normal conditions

* A valuable method recently published by Mallory in *The Journal of Experimental Medicine*, ii (1897), 531. The dye has a special affinity for neuroglia fibres, and stains also nuclei and to a slight extent protoplasm of neuroglia cells. Its results are more satisfactory in our hands than those gained by the phospho-molybdic-acid-hæmatoxylin method. Other uses than that for neuroglia do not at present concern us.

by the same method of staining. Very striking and characteristic are fibres of wide calibre, which are plentifully seen throughout the section and to which the epithet "hypertrophied" may properly be applied. Such coarse fibres are frequently seen to be undergoing retrograde changes suggesting hyaline degeneration, characterized by local swellings and slight alteration in staining reaction. The nuclei (neuroglia cells) of the tumor proper are, as already stated, insignificant in number; in shape they show a considerable degree of variation, an elongated form is frequent. Certain cells, not frequently met with, are of special interest. Studied by the phospho-molybdic-acid-hæmatoxylin and phospho-tungstic-acid methods, they show usually at one pole of an elongated cell body a process first of wide calibre and gradually narrowing toward its extremity, finally becoming a fibre similar in appearance to the differentiated form. So far as one may determine from this staining method such processes stand in direct relation to the cell protoplasm, and are therefore to be regarded as undifferentiated fibres. In many cases the difficulty of determining the relations of cells and fibres is extremely great. Typical "Astrocytes," as described by Stroebe, are not discoverable. In spite of the large size of the fibres and the ease with which each may be followed, no evidence whatever of branching is to be found. The tumor, excepting at its point of transition to normal brain tissue, contains no myeline fibres (Plate XLVIII, Fig. 3). Ganglion cells do not occur.

Microscopically, as well as macroscopically, the tumor is sharply circumscribed, though not encysted. Its character remains unchanged until within a short distance of its transition to normal tissue, excepting for the finer calibre of the neuroglia fibres. Indication of transition is given by the appearance of an occasional partially degenerated myeline fibre (Plate XLVIII, Fig. 3). A slight infiltration of the white matter occurs, but it is much less in degree than is ordinarily the case. Very striking, however, is the altered character of the neuroglia at this slightly infiltrated point. The fibres are of fine calibre and it is quite impossible to determine absolutely whether or not they stand in direct relation to the cell protoplasm. The appearances in some instances are exceedingly deceptive, and a most conscientious examination leaves one undecided as to the actual condition. Evidently the proliferation of the neuroglia is here actively going on, and, although we venture no dogmatic opinion, the appearances on the whole justify us in the statement that in many instances we probably have attenuated protoplasmic processes which are as yet undifferentiated in Weigert's sense. An exceedingly instructive picture is afforded by a thin section stained with phospho-tungstic-acid-hæmatoxylin, though we do not pretend to say that it gives conclusive

information in support of one or the other of the opposing views regarding the structure of the neuroglia (Plate XLVIII, Fig. 4).

In brief, we have microscopically a circumscribed tumor composed largely of newly formed differentiated neuroglia fibres without marked cellular proliferation, to which the diagnosis Glioma durum may be properly applied.

There are evidently certain points of special interest in this case, to which we here make brief allusion in view of a complete discussion to follow. 1. Its macroscopic appearance, circumscribed and associated with extensive cyst formation. 2. Its microscopic structure, dense, consisting chiefly of differentiated fibres, rather than of cells. 3. The presence in it of certain cells, possibly belonging to a transitional form. To the second and third of these points we shall devote particular attention.

Upon the general subject of glioma, the researches of the past few years have thrown much light, and that from various directions, so that a statement of our present position on certain disputed points is desirable. Embryological research and new staining methods have together done much toward overthrowing old conceptions and bringing a degree of scientific exactness into our knowledge of normal and pathological neuroglia.

The recognition through the studies of W. His, Cajal, von Lenhossék and others of the ectodermal origin of the neuroglia has of necessity led to a fundamental change in our conception of that tissue, both in its normal and pathological conditions. In speaking of those pathological processes peculiar to the nervous system, *e. g.*, the so-called scleroses, the term connective tissue should be absolutely dropped as unnecessary and misleading in its significance, in that it suggests a tissue of mesoblastic origin. The small part which mesoblastic connective tissue plays in the reparative or compensatory processes occurring in the central nervous system is by degrees being recognized, and the use of the term in describing pathological changes is less often, though still frequently, met with. We must clearly understand that neuroglia, from the histogenetic point of view, has no affinity whatever with connective tissue, and also the second fact, upon which Weigert insists, that its function for the nervous system is precisely that which belongs to the connective tissue

in the case of other organs. With these two points in mind, much of the vague phraseology everywhere met with might easily be avoided. Until the neuroglia is recognized as an epiblastic tissue, possessing, however, the functional and anatomical significance of mesoblastic connective tissue, we shall hardly reach that accuracy in speaking of the pathological changes in the nervous system which is so clearly desirable.

Our purpose in bringing up this point is a definite one, with particular reference to a matter which is ripe for discussion, viz., the relation of the gliomata to the sarcomata, or, put more broadly, the relation of epiblastic new growths to mesoblastic new growths. It is clearly evident that there is much confusion both of a theoretical and practical sort regarding these two classes of tumors. The confusion has arisen from two chief sources; first, the failure to recognize clearly the epiblastic origin of neuroglial tissue, and, secondly, the inadequacy of staining methods largely in vogue, *e. g.*, hæmatoxylin-eosin, to determine histological distinctions of a fundamental sort between neuroglia and connective tissue, or between the cells of gliomatous and sarcomatous new growths. So long as the development of the nervous system remained unstudied it was natural that the neuroglia should be regarded as an ordinary connective tissue of mesoblastic type, and as a consequence that pathologists should include the gliomatous tumors among those of the connective tissue class. Hence the inevitable confusion which has prevailed and still exists to a certain degree regarding a proper classification. Particularly noticeable is this fact in the continued use of the term "glio-sarcoma" as descriptive of a mixed growth, in which both gliomatous and sarcomatous elements are conceived as growing side by side. Such a nomenclature was clearly natural when neuroglia was regarded merely as a connective mesoblastic tissue of peculiar character. With the growth of our knowledge concerning its epiblastic origin the anomaly of a mixed mesoblastic and epiblastic tumor, bearing the name glio-sarcoma, becomes apparent, and has already been the subject of considerable discussion; nevertheless the term glio-sarcoma still maintains its place in most of the text-books as a definite form of new growth.

It will be of interest to pass quickly in review some of the expressed conceptions of gliomatous and so-called glio-sarcomatous tumors by pathologists of recognized authority.

Orth,* after speaking of gliomata, glio-sarcomata and sarcomata as distinct forms of tumor of the brain, goes on to say: "Ist nur das Faserwerk vorhanden, so liegt ein reines Gliom vor, sind viele freie Zellen dabei, so wird man ein Gliosarcom diagnosticiren."

* Orth, Pathologisch-anatomische Diagnostik, pp. 116, 117. Berlin, 1894.

Thoma* lays stress upon the epiblastic origin of the gliomatous tumor, but admits the possibility of such tumors going over into quickly growing glio-sarcomata: "in rasch wachsende Gliosarkome übergehen."

Birch-Hirschfeld† allows the term glio-sarcoma and distinguishes sarcoma from glioma, "durch rascheres Wachsthum, reichliche Entwicklung der zelligen Elemente und bedeutendere Grösse derselben"—evidently not differences of a fundamental sort.

Ziegler‡ faces the difficulty and after criticising the term glio-sarcoma as ordinarily used, says: "Ein wahres Gliosarkom kann dadurch entstehen, dass im Gliom eine perivasculäre, adventitielle Zellwucherung auftritt deren Product einen integrirenden Bestandtheil der Geschwulst bildet."

Stroebe§ admits the possibility of mixed forms, but would limit the term glio-sarcoma, as Ziegler does, to gliomatous tumors with sarcomatous growth starting from the blood-vessels. Such a distinction as that made, for example, by Birch-Hirschfeld, he would discard.

Without discussing the matter at length, Oppenheim|| admits the occurrence of mixed tumors in which sarcomatous tissue and gliomatous tissue alike appear. Starr¶ likewise uses the term. Strümpell, on the other hand, in the last edition of his "Specielle Pathologie und Therapie," makes no mention of glio-sarcoma as a distinct form of tumor.

Evidently from the few examples above cited the term is one deeply rooted in the literature and regarding which much confusion exists. Few writers are willing to give it up entirely, though in certain cases modifying it to a degree which amounts to its complete renunciation (Ziegler, Stroebe). As popularly employed and used by writers on classification of tumors the word is manifestly unfortunate and misleading. Lenhossék** states clearly the absurdity involved when he says that from the histogenetic point of view—and that is the only one we may properly use in such a matter—it is impossible that a sarcoma should at the same time be a glioma, since their cells are differently derived, in the one from mesoblast and in the other from epiblast. It is conceivable that a glioma and a sarcoma might grow side by side, but we have no reason to suppose that this ever actually occurs, nor do we have any analogy to such a process in other forms of new growth.

Considering the inadequacy of previous methods to determine the true

* Thoma, Lehrb. d. path. Anatomie, Th. I, pp. 661-663. Stuttgart, 1894.
† Birch-Hirschfeld, Lehrb. d. path. Anatomie, Bd. ii, pp. 332-334.
‡ Ziegler, Lehrb. d. path. Anatomie, Bd. ii, p. 363.
§ Stroebe, op. cit.
|| Oppenheim, op. cit., p. 10.
¶ Starr, Brain Surgery. New York, 1893.
** Lenhossék, op. cit., p. 245.

character of the component cells of such growths, and also the pathological anomaly involved in the term, we are justified in excluding the class of so-called glio-sarcomata from the brain tumors. In Ziegler's and Stroebe's sense, the term may be admitted, viz., glioma with sarcomatous elements derived from proliferation of perivascular connective tissue, but with the old confusion still in our minds it would unquestionably be better to drop the term entirely, as Strümpell apparently has done. Unquestionably the essential character of a new growth in the brain, as elsewhere, is either epiblastic or mesoblastic, and it is certainly conducive to clearness, in the present state of our knowledge, to speak of it only from the point of view of its characteristic histological element.*

Reference has already been made to Stroebe's detailed paper on glioma cerebri. Its interest and importance lie chiefly in the use of a special method of staining, Mallory's phospho-molybdic-acid-hæmatoxylin, and the somewhat far-reaching conclusions drawn therefrom. The method as originally given out by Mallory† was designed chiefly as a neuroglia stain, but in no sense as a selective one, the protoplasm, nucleus, and processes of neuroglia cells staining sharply as well as other elements, *e. g.*, axis-cylinder processes. By its use a chemical differentiation of neuroglia fibres, as attained by Weigert's and Mallory's modified fibrin stain, was clearly impossible. As a result of the sole use of an inadequate method, Stroebe finds the neuroglia fibres to be, in every case, processes of neuroglia cells and places himself in direct opposition to Weigert, who maintains the definite disassociation of fibres and cells in developed neuroglial tissue. At a discussion in the pathological section of the Naturforscherversammlung,‡ held at Frankfurt in September, 1896, Stroebe and Weigert have reiterated their distinctive views and are still opposed on this most fundamental histological point.

As is well known, previous to Weigert's monograph the general conception of the neuroglia was one which the Golgi methods had done much to strengthen, namely, that neuroglia fibres were direct outgrowths of cells, and in no way to be regarded as an intercellular substance. The various diffuse staining methods had tended to make such an idea unassailable. With the appearance, however, of Mallory's and Weigert's publication of a differential stain for the neuroglia an entirely new light was thrown upon the matter, leading to Weigert's dogmatic position of the disassociation of fibres and cells in adult neuroglia. He bases his atti-

* A similar position has been taken by Eurich. *Brain*, spring and summer, 1897, 114.

† Mallory, *Anat. Anzeiger*, vi (1891), 375.

‡ *Centralbl. f. allg. Path. u. path. Anat.*, vii (1896), 864.

tude chiefly on the fact that since the protoplasm of neuroglia cells does not stain and the neuroglia fibres do, a chemical difference of a fundamental sort must exist between cells and fibres, and therefore that fibres reacting in a definite way to a chemical stain cannot stand in the relation of processes to protoplasm which reacts in an entirely different way. Stroebe cleverly answers this argument by saying that because we have Nissl granules, for example, or neuraxon processes staining differently chemically, we do not therefore maintain that they are not integral parts of the cells in which they lie or from which they proceed. In such a case there is no thought of an intercellular substance. Unquestionably this point is well taken, and Stroebe's further suggestion is also pertinent, that the question of spatial (*räumlich*) relation of cells and fibres is of importance, a relation upon which Weigert's method throws no light, since one element, viz., the protoplasm, necessary for the determination of such spatial relations, remains unstained. This relation the phosphomolybdic-acid-hæmatoxylin method of Mallory supplies, Stroebe maintains, and that by it the existence of fibres as processes of cells may be established. It is quite beyond the scope of this paper to enter into the various arguments and facts which Weigert advances in support of his view, nor can we discuss Stroebe's position at length. We must content ourselves with as brief a statement as possible of facts which are accepted, and also of those which remain a subject of dispute.

We may regard as established that: 1. There is a definite chemical difference between neuroglia fibres and the protoplasm of the neuroglia cells. Such a conclusion the Weigert-Mallory method does not permit us longer to doubt. 2. There is an enormous development of fibres in adult neuroglia, which bear no constant relation to the cellular elements. 3. Whatever the relation of cells and fibres may be, the neuroglia acts within normal and pathological conditions as a connective tissue, in spite of its epiblastic origin. To Weigert chiefly we owe the definite establishment of these facts.

Points still in dispute are: first and most important, the relation of fibres to cells; and, secondly, if fibres are completely differentiated (Weigert), how and at what stage of their development do they become so; and as a corollary, do we find cells which may be regarded as transitional forms? It is not necessary to reiterate what a study of published articles on the subject forces upon us as established. Our purpose is rather to take up in certain detail the points recog-

nized as still doubtful in the light of the somewhat unique tumor of which we have already given a description, and also of another growth which we must likewise include in the class of gliomata. Allusion has already been made to features of particular interest, in the case whose description has been given. Of chief significance for our present discussion is the enormous development of fibres, as compared with cells. In this fact alone unquestionably lies a strong argument in favor of Weigert's view. It is difficult to conceive that fibres of such numbers and density of arrangement and calibre could, from a mere mechanical point of view, be processes of the cells found in their midst. Nor would Stroebe's argument hold here, that fibres apparently passing by cells may have had their origin from distant cells, since in this tumor we have a circumscribed growth, in no part of which cells preponderate sufficiently to give such a view a reasonable foundation. One's unavoidable impression in studying the growth histologically is that the fibres are essentially distinct from the cells, and that thus far we have a substantiation of Weigert's dogmatic view.

But just here a further question arises, which, so far as it is possible for us to learn, has received much less attention than its importance warrants. Admitting with Weigert that the essential feature of the neuroglia here developed is fibres, does it necessarily follow that all the fibres are differentiated, and represent therefore an intercellular substance? Does not Weigert take a step beyond the knowledge furnished by his method when he makes so sweeping an assertion? May there not, even in developed neuroglia, be a certain number of neuroglia elements, whose fibres are still in a relation of physical continuity with cells? If such a possibility be admitted, as we believe it must be, is not Stroebe entirely right in maintaining that Weigert's method is entirely incapable of giving us that information? Must we not appeal to a more diffuse method, in which protoplasm is also stained, in order that a physical continuity of fibre and cell protoplasm may be established, if such exist?

Stroebe, using chiefly Mallory's phospho-molybdic-acid-hæmatoxylin, while admitting as a possibility the complete differentiation of fibres, is sceptical that it exists to any considerable degree, and is tenacious of the old idea of cellular processes. Why is not a middle position between the two extremes as represented by Weigert and Stroebe reasonable and

probable? For the establishment of such a conservative position the demonstration of transitional forms of cells is required. About such cells peculiarly little has, up to this time, appeared in the literature. There certainly is no *a priori* argument against the possibility of the occurrence in the same growth or in different growths of differentiated fibres and fibres still in relation to the cells as direct processes from these cells. That Weigert's method does not demonstrate such transitional forms does not argue against their existence, since the method confessedly could in no case prove their existence. In short the proper investigation of the question demands the use of more than one method, in the same sense that a study of nerve fibres demands the employment of a neuraxon stain combined with a myeline stain. In justice to Weigert it is to be stated that his work heretofore has dealt largely with normal adult neuroglia, in which he regards the disassociation of cells and fibres as complete. Any departure from this rule applies only to embryonic periods of growth. At some time in the development of neuroglia Weigert therefore tacitly admits the association of cells and fibres as cell processes, but passes over any discussion of the manner of their differentiation. In pathological conditions just here lies a point of much practical and theoretical importance. In a growing tumor we should certainly expect to find cells with associated fibres in conjunction with fibres absolutely differentiated. Such transitional forms for Stroebe have little significance, since he does not admit an ultimate differentiation; by Weigert they are passed over without detailed discussion as embryonic cells which have no place in adult neuroglia. On page 106 of his monograph, for example, he says, speaking of the opinion of previous writers, "dass die Neuroglia nur aus Zellen und deren Fortsätzen besteht so trifft dies beim Menschen nur für die Embryonalzeit zu."

Taking a position between these two extreme views, it seems altogether reasonable to suppose that in a slowly growing tumor we should find both differentiated and undifferentiated fibres. For the investigation of this question the tumor above described is peculiarly adapted, since in just such a growth, with an enormous preponderance of differentiated fibres, we should be least likely to discover cells of a transitional type. If found, therefore, we should be justified in attaching a distinct significance to their presence.

A painstaking study of sections with the end in view of discovering such cells yields, in our opinion, positive results, though, from this

growth alone, not so conclusive as might be desired. Weigert's recent method, as Lenhossék and Stroebe have shown, is valueless in this investigation, since its function is to stain differentiated fibres. Mallory's phospho-molybdic-acid-hæmatoxylin and the most recent phospho-tungstic-acid method, also of Mallory,* are, on the other hand, suitable, since they color protoplasm in addition to nuclei and fibres. Careful search with high powers shows in those parts of the tumor where the development of fibres is less dense an occasional elongated cell, somewhat pear-shaped (see general description of microscopic appearances), from one pole of which comes off a process, gradually tapering in calibre and staining with phospho-molybdic acid, in a way similar to the protoplasm of the cell body. The conclusion is justified that such fibres are direct outgrowths of the cells. It is possible and probable that such processes would not stain by the Weigert or Mallory fibrin method, owing to certain chemical peculiarities. It is certain that their relations to the cells could, in any case, not be demonstrated by its use. Typical astrocytes, as described by Stroebe and admitted by Weigert to exist in rapidly growing gliomata, we are unable to detect. These cells, which we think it reasonable to speak of as transitional forms, are rather unipolar or bipolar in character.

In such a tumor as the one hitherto discussed, evidently slow growing and made up chiefly of differentiated fibres, these transitional forms, if such they be, are infrequent, as we should expect them to be. It is therefore of much interest to turn to the study of a tumor (Case II) belonging to the same general class, but of a distinctly different type, viz., a rapidly growing glioma with undifferentiated fibres and proliferation of perivascular connective tissue.

A nuclear stain—hæmatoxylin-eosin—shows the tumor to be made up of cells closely packed and varying to a considerable degree in size. The larger cells are easily seen to be associated with the vessel walls; they are evidently proliferated, and karyokinetic figures are frequent. The mass of the growth, on the other hand, is made up of smaller, densely crowded cells, not distinctive in character by this method of staining. The use of Mallory's modified fibrin method affords absolutely no infor-

* *The Journal of Experimental Medicine*, ii (1897), 531-2.

mation regarding the presence of neuroglia fibres, nor does Mallory's phospho-molybdic-acid-hæmatoxylin in an unteased section. On teasing a small bit of the tumor, however, and then staining with phospho-molybdic acid, cells of a strikingly interesting sort are revealed (Plate XLIX, Figs. 5 and 6). They are small, provided with a fair amount of protoplasm, from which usually, at opposite poles of the elongated cell body, come out processes tapering to fine fibres, which form a close-meshed network without discoverable anastomosis. There can be no question that these fibres are direct outgrowths from the cell body, nor is it open to reasonable doubt that they represent undifferentiated neuroglia fibres. None of the cells, however, are of the Deiters' "spider" variety.

This second tumor, therefore, is a rapidly growing, cellular glioma, with undifferentiated fibres; or, to use a convenient, if not a scientifically exact term, a glioma made up of cells of a transitional type. Differentiated fibres in Weigert's sense, as intercellular substance, are not discoverable and undoubtedly do not exist. That the fibres described as cell processes might later have become differentiated and then stained by the Weigert method is probable. This tumor,* therefore, is to be regarded as belonging to the text-book variety usually described; it derives its interest for the present discussion from the study of its individual cells in teased preparations. It will be seen that the cells of which this growth is primarily composed resemble closely the few cells already described as occurring in Case I. This is a matter of significance and justifies the conservative position which we have already indicated as, in our opinion, the correct one. In short, it may be thus stated: in glioma, neuroglia consists of cells with undifferentiated fibres and of cells together with differentiated fibres, the predominance of differentiated fibres depending chiefly on the age of the growth.

In other words it is not possible to dogmatize as to the relation of fibres and cells in any given case, since a range of variation is clearly possible between the extremes represented by Stroebe on the one hand and Weigert on the other. Case I is an almost conclusive argument in favor

* This is, in general, a variety of tumor to which the term glio-sarcoma has frequently been applied.

of Weigert's view. Case II, just alluded to, is equally suggestive of the correctness of Stroebe's conception.

It is hard to see why it is so difficult to conceive of the evolution of neuroglia from cells without processes to cells with processes, and then to cells whose processes have been completely differentiated into fibres. Could this be accepted as the natural history of the process, the controversy would seem to be at an end; that it is *a priori* a reasonable explanation is evident; that it is supported by a certain amount, at least, of anatomical evidence, the study of the foregoing varieties of gliomatous tumors indicates.

Admitting the correctness of this view, another point of interest at once arises, viz., the possibility of diagnosticating differentially the sarcomata and gliomata at all stages of their growth. The term glio-sarcoma has been sufficiently abused, as we have attempted to show. In the future, glioma or sarcoma or "unable to determine" should take the place of the straddling glio-sarcoma. In what cases, then, is it possible that a differential diagnosis may be difficult or impossible? Evidently in those, and only in those, in which the component cells have not attained a growth sufficient to designate them absolutely as glia cells, or, in other words, to differentiate them from connective-tissue or sarcoma cells. In the present state of our knowledge, and if the ideas already expressed be correct, we must admit that transitional forms may occur which might lead to confusion as regards the processes of the cells. We have already shown, following Stroebe, that Weigert's fibrin method is inadequate in this question of diagnosis, for determining the character of the cellular elements at certain stages of their growth, viz., while the fibres are still processes of the cells. The phospho-molybdic-acid, or better, the phosphotungstic-acid methods render here, for reasons already given, more definite information. With a conscientious study of any growth by the various means now at our disposal an error is not likely to occur. Essential for a diagnosis of glioma is the demonstration of cells and fibres—the fibres being either still associated with the cell protoplasm or differentiated from it, as in older forms of growth. In the latter case Weigert's and Mallory's recent methods can leave no doubt as to diagnosis, from their inevitable staining of characteristic fibres. In the former case those methods evidently do not prove the tumor to be other than glioma from their failure to stain fibres, since these fibres at this stage of their growth are only stainable by other methods. In a case where there is still doubt after the staining of cut sections, teasing is desirable and may, as in our second case, give the desired information. We are of the opinion that macroscopic appearances, although in general characteristic,

are nevertheless an unsafe guide, and that in doubtful cases recourse should always be had to the microscope. A complete and interesting discussion of this question from all points of view is to be found in Stroebe's already frequently quoted paper. In general, he has undoubtedly brought out the points of chief importance in the differential diagnosis. We must, however, take direct issue with him in one of the general conclusions to which his study has led him. After speaking somewhat in detail of the possibility of confusing with glia cells certain forms of sarcoma cell, and furthermore the possible confusion arising from the staining of fibrin threads, he concludes: "Immerhin ergiebt sich aus den letzt erwähnten Befunden die Forderung, dass zur Diagnose eines Glioms nicht nur die Anwesenheit eines Fasernetzes zwischen den Zellen beobachtet sein muss, sondern dass auch die Verbindungen der characteristischen Fasern mit den Zellen in Gestalt der eigenartigen, vielstrahligen Gliomzellen festgestellt sein muss." * In the light of our first case there can be no doubt that the above statement is incorrect. Characteristic in this tumor is the very fact which would lead Stroebe to exclude it from the class of the gliomata, viz., the presence of fibres which cannot be shown to stand in direct relation to the cells. But that the tumor is a glioma there can be absolutely no question; it is equally evident that the fibres do not stand to the cells in the relation which Stroebe holds as essential. In other words, we have a glioma with a preponderance of free fibres. Stroebe's error here, as we think elsewhere in his discussion, lies in the use of a method from which he draws too far-reaching deductions.

To a certain degree Weigert apparently agrees with Stroebe in his conception of the essential character of a gliomatous tumor. After speaking of "the fable of softened central gliosis," met with in the teaching of syringomyelia, and the general confusion arising from our previous ignorance of the marked collection of glia about the central canal, Weigert goes on to lay down the following broad distinction: "Die Verwirrung wurde noch dadurch vergrössert, dass man 'Gliose' d. h. krankhafte Vermehrung der Neurogliafasern mit 'Gliom' verwechselte. Bei den Gliomen sind die Gliafasern nicht vermehrt, sondern die Gliazellen. Ja nicht nur das, sondern diese letzteren verlieren zum grossen Teile die Fähigkeit, abgesetzte Fasern zu erzeugen, und *bleiben in ihrem ursprünglichen protoplasmatischen Zustande. Man darf sich daher nicht wundern, wenn man gerade in Gliomen e c h t e Deiterssche Zellen findet, wie im Embryo.* Das Verhältnis der Gliome zur Gliose ist also, wie das des

* Op. cit., p. 459.

Sarkoms zur entzündlichen Bindegewebswucherung oder wie zum Fibrom." *

Weigert is said to have previously expressed an opinion somewhat at variance with the above statement, in that he admitted that numerous glia fibres might be found in true gliomatous growths, an implication, at least, of an increase of fibres.† In his later statement on this point, already quoted, there is no room for doubt that he makes an essential distinction between glioma and gliosis, holding that the former shows an increase of glia cells and not of glia fibres, whereas in the latter condition there is a pathological increase of neuroglia fibres.

In the light of the histological study of the tumor which forms the basis of this paper, this position of Weigert's is completely untenable for the same reason that we believe Stroebe's view untenable. We are dealing in our first case with a tumor—a glioma—and not a gliosis, and yet unquestionably we have a "krankhafte Vermehrung der Neurogliafasern," which, according to Weigert, is characteristic only of gliosis. On the other hand the glia cells are not increased, which again, according to Weigert, should invariably occur in glioma. Hence in this tumor we have what Weigert would term a gliosis, evidently an absurdity, since it is inconceivable that a glioma should at the same time be a gliosis, if any distinctive meaning at all is to attach to either term. The only possible position for us to take is one quite opposed to Weigert's, in that we fail utterly to recognize his distinction as fundamental. The teaching of the tumor under discussion unquestionably is that a pathological increase of neuroglia fibres is not inconsistent with the term "glioma." It is to be mentioned that at the discussion between Weigert and Stroebe at Frankfurt‡ the former is reported to have made the following statement: "Es sei zuzugeben, dass in Gliomen ausser den Zellen auch die Fasern vermehrt sein können," with the implication that such fibres do not readily stain owing to the imperfection of the method. In any case here is a direct contradiction of the previous statement quoted that the glia fibres are not increased in glioma. We are therefore at a loss to know what Weigert's real position in the matter is, since he has apparently modified the unequivocal and dogmatic statement contained in his monograph. Admitting the incorrectness of the first statement, a further detailed criticism of the assertions following is hardly necessary. There can be no doubt, judging from our tumor, that in glioma the glia cells

* Loc. cit., p. 156-157. The italics are Weigert's.

† Quoted from Lubarsch-Ostertag, Ergeb. d. allg. Path. u. path. Anat., Abth. ii, 1895, p. 339.

‡ Loc. cit.

are capable of producing differentiated (*abgesetzte*) fibres, and that they distinctly do not in all cases remain in their original protoplasmic condition. The presence of true Deiters' cells is, according to our view, possible, but that the tumor should be formed wholly of such cells as Weigert implies does not follow, and in our case is not true.

Finally Weigert's analogy that the relation of glioma to gliosis is similar to that of sarcoma to inflammatory connective-tissue growth or to fibroma is inexact. The glioma under discussion, for example, has a much closer resemblance to a fibroma than to a sarcoma. In short Weigert's too dogmatic distinction does not hold. Though in general true, it is incapable of serving as a fundamental means of distinguishing glioma from gliosis. Whatever other points are doubtful, of this the study of the tumor before us can leave absolutely no question. Weigert has but added to the prevailing confusion by asserting the existence of a distinction, of which he himself has later become doubtful, and which we must certainly regard as totally unessential to the real question at issue.

It is not our purpose to speculate as to fundamental pathological distinctions in the present imperfect state of our knowledge regarding the neuroglia, but the suggestion is unavoidable that here as elsewhere in the nervous system we may find that the whole matter is much simpler than we now suppose, and that the various conditions which we conveniently designate by separate names may in ultimate analysis be the same process manifesting itself diversely, as excited by causes which lie quite beyond the present field of inquiry. In short, it is much more profitable to study what glioma and gliosis, for example, have in common, than to seek for distinctions which analysis is likely to show to be not fundamental. Clinically, glioma, as a new growth, may be advantageously distinguished from gliosis as evidently giving rise to an entirely different symptom-complex, but to maintain such distinctions pathologically is altogether detrimental to progress, unless histological evidence bears them out. As yet we have no adequate evidence which permits us to assert that gliosis and glioma are sharply differentiated processes; they are evidently in many respects closely analogous and, so far as our knowledge at present reaches, their differences lie rather in their methods of growth than in any fundamental histological peculiarities.

Whatever of value the foregoing discussion may have lies in two chief factors: first, in the peculiar histological structure of the tumors studied; and secondly, in the varied methods used in their investigation. As the first case goes far to substantiate Weigert's general

view, so the second case, less completely described, is equally strong evidence in favor of Stroebe's conception. Conversely, however, the first case is subversive of Stroebe's idea, as is the second, partially at least, of Weigert's. The two tumors, therefore, are of particular interest from the fact that they show neuroglia proliferation at two distinct phases of growth hitherto, we believe, undescribed in tumor formation. It is a perfectly logical deduction, if not an absolutely demonstrable fact, that the differentiation of neuroglia fibres occurs in the course of growth, if, as in these cases, we can show with a fair degree of certainty that at one stage, presumably an early one, the fibres are still associated with the cell protoplasm as processes, while at another and later stage they are completely differentiated and become neuroglia fibres in Weigert's sense. Any other hypothesis of the differentiated fibres would imply, as Dr. Mallory has suggested to me, that at some period of growth the elongated protoplasmic processes cease to exist as such and disappear, to give place to other and separate fibres of unknown origin. Unquestionably the idea of a gradual differentiation is more consistent with nature's economy, as well as with certain facts of observation which we have attempted to bring forward. It is evident that the transitional forms are of the utmost interest and importance in the establishment of such a view; and just here we find, as before suggested, the gap in our knowledge which the work of the future should fill; it is hoped that this investigation may have at least the effect of indicating a profitable line of investigation.

The views of Stroebe and Weigert evidently represent the horns of a real dilemma, whose solution must depend upon a careful weighing of the evidence by all the means at our disposal. Even when that is done, it is probable that our ultimate conclusions must in a measure rest upon other data than those of direct observation. For the rigid adherent of either Weigert's or Stroebe's views there will always be the possibility of escape, in that our present means of investigation do not permit of absolute dogmatism on either side. Nevertheless, in view of all the facts, we feel altogether justified in maintaining the conservative and natural attitude that the dilemma

probably, in last analysis, does not exist, since both its extremes are true in part but not completely. Such, at least, is to us the teaching of the pathological glia which we have described in the two tumors chiefly under consideration.

CONCLUSIONS.

The results and conclusions of the above investigation are:

1. The term glio-sarcoma should be dropped, as unscientific and misleading in its significance.

2. The problems regarding neuroglia demand varied methods for their adequate study.

3. With all the means at our command, the absolute determination of the relation of cells and fibres in individual cases remains difficult and at times impossible.

4. No criterion has yet been offered to determine a fundamental distinction between glioma and sarcoma (Stroebe); and secondly, between glioma and so-called gliosis (Weigert).

5. The development of neuroglia in all probability is from cells with protoplasmic processes to cells with differentiated and independent fibres.

6. Herein lies a possible reconciliation of the conflicting views concerning the ultimate structure of human neuroglia.

DESCRIPTION OF PLATES XLVI-XLIX.

Plate XLVI.

Frontal section—actual size—through tumor at its point of greatest development. Ventricular cavities are seen to be distinct from the cysts surrounding the tumor proper. Chief destruction has taken place in the left hemisphere.

Plate XLVII.

Fig. 1.—Section through tumor from Case I. Mallory's modified fibrin stain. Leitz oc. 3; obj. 3. Showing fibre structure; probable lymph spaces containing desquamated cells; preponderance of fibres over cells.

Fig. 2.—Section through same. Mallory's modified fibrin stain. Zeiss oc. 4; obj. $\frac{1}{12}$. Showing fibres varying in size and occasional nuclei of neuroglia and desquamated cells.

Plate XLVIII.

Fig. 3.—Section at edge of tumor from Case I. Weigert's rapid copper-hæmatoxylin method. Leitz oc. 3; obj. 5. Showing partially degenerated

myeline fibres. The background represents the infiltrating gliomatous tissue.

Fig. 4.—Section at edge of tumor from Case I. Mallory's phospho-tungstic-acid-hæmatoxylin method. Zeiss oc. 4; obj. $\frac{1}{12}$. Showing neuroglia cells and fibres. In this section is shown the difficulty of stating dogmatically whether or not certain fibres stand in direct relation with cells. The fibres here are finer and the cells more numerous than in sections taken from the central portions of the growth representing an older phase.

Plate XLIX.

Fig. 5.—Teased preparation from Case II. Mallory's phospho-molybdic-acid-hæmatoxylin method. Leitz oc. 3; obj. 5. Showing cells with long processes and no differentiated fibres. The cells are for the most part unipolar or bipolar in character, and no doubt are young neuroglia cells.

Fig. 6.—Same. Zeiss oc. 4; obj. $\frac{1}{12}$. Selected cells, showing character of processes. It is to be noted that the nuclei are large proportionately to the amount of protoplasm.

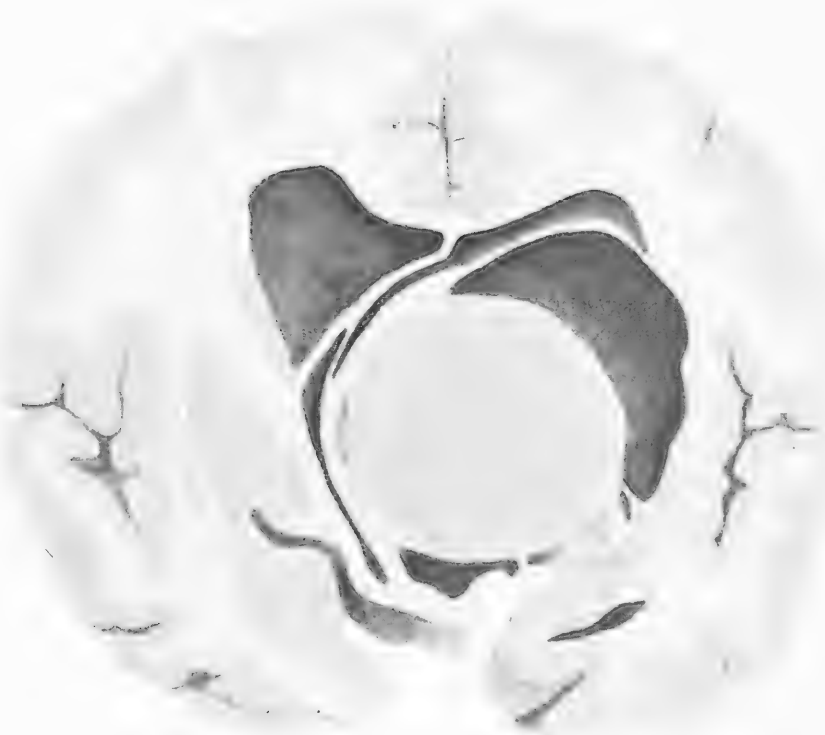


FIG. 1.

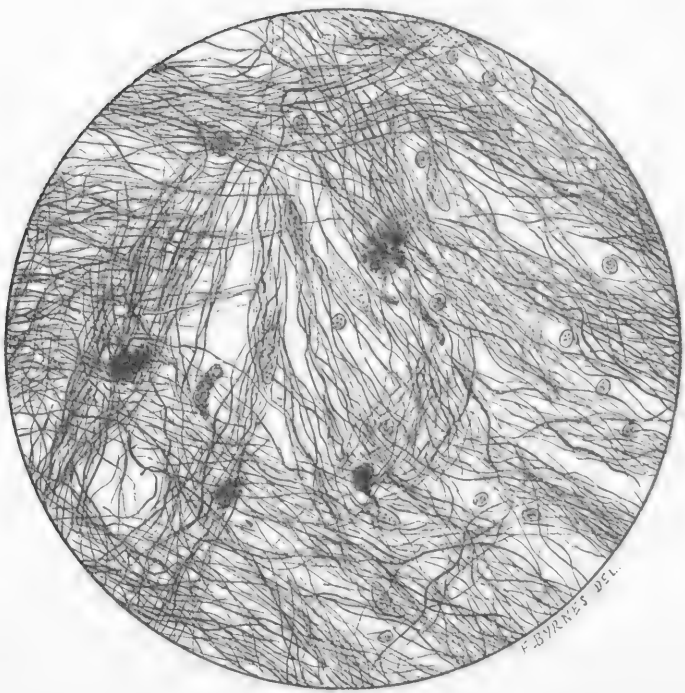


FIG. 2.

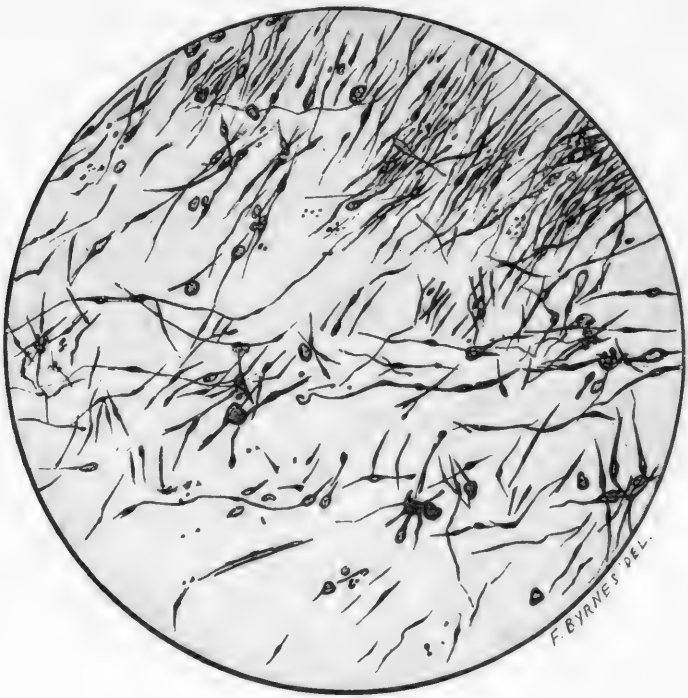


FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

THE CLINICAL COURSE AND PATHOLOGICAL HISTOLOGY OF A CASE OF NEURO-GLIOMA OF THE BRAIN.

By H. M. THOMAS, M. D., and ALICE HAMILTON, M. D.
(From the Pathological Laboratory of the Johns Hopkins University and Hospital.)

Plates L–LIII.

Part I.

The Clinical Course.

By H. M. THOMAS.

Clinical Summary. Male; aet. 38; unmarried. Excellent family and personal history. Syphilis denied. Onset in 1890. Convulsive seizures, beginning in toes of right foot, followed by loss of consciousness. Attacks every few weeks for six years, all beginning in right foot and often involving right arm. Loss of consciousness, common at first, was absent during the last three years. Gradually increasing weakness of right leg for two years. Paralysis of arm during observation. No headache, vomiting or other general symptoms. Examination, autumn, 1896. Hyperæmia of the optic discs (no neuritis). Altered percussion note over left parietal region. Distended veins on left side of forehead. Slight weakness on right side of face. Distinct paralysis of right arm and leg. Increased reflexes and marked muscular atrophy in right limbs. Disturbance of temperature sense in right lower leg. Exploratory operation. Bone meninges normal; cortex inexcitable by strong faradic current. Death twelve days after operation.

The patient was an unmarried man, 38 years old,* who complained of weakness of the right leg, and of convulsive attacks, localized in this leg. The family history contained nothing of significance. In the personal history there was nothing that seemed to stand in a causal relation to the trouble from which he was suffering. The only injury that he had received was at the age of eight years, when he fell ten feet, striking the

* Clinic for nervous diseases, Disp. No. 7556. Hospital, Med. No. 6721, Surgical No. 5923.

back of his head. He was not unconscious, but was able to get up immediately. He denied ever having had any venereal disease.

The trouble for which he sought relief had its onset in May, 1890, *i. e.*, six and a half years before his first visit to the Dispensary. For a year previous to this time he had been very much confined by his work, which was that of a book-keeper, and had suffered more or less from headaches which he attributed to constipation and to lack of outdoor exercise. He considered himself, however, in good health. In May, 1890, during a vacation of a week, he had his first attack. This occurred while he was at work painting the mast of his sailboat. At this time he had a sense as if something were going to happen, and he felt that it would be better for him to get down and work upon the bottom of the boat. He thought he had been dizzy, and soon forgot about it; in about half an hour, however, he began to have a sensation of numbness in the great toe of the right foot. This increased until it felt as if some one had hold of the great toe. He then became unconscious. He was alone in the boat, and was unable to tell how long the attack lasted. Upon regaining consciousness he discovered that he had bitten his tongue. He went to the club house, dressed, dined with a friend, and felt in no way incapacitated. The next morning, after a good night's rest, he returned to work on his boat, but upon lifting a heavy weight he became unconscious and fell. In this attack there seemed to have been no premonitory symptoms. He was told by his friends that during the same day he had had three or four convulsions. Of these he had no clear recollection. The next morning he had a slight attack in which he did not lose consciousness. This one began in the toes of the right foot, and later affected the leg. After this he went away to the mountains and was quite free from attacks until August, when he had, after the exercise of climbing a mountain, another convulsion. This also began in the right foot, which he stated was contracted for at least a minute before he lost consciousness. That same evening he had another attack, beginning in the same way. After this there was another interval of three months in which he had no convulsions. They then recurred, and he had several within a few hours. From this time on he had an attack on an average of about once every three weeks, during the years of 1891, '92, '93 and '94. In 1895 and '96 they became somewhat less frequent, occurring at intervals of about a month.

All the attacks began in the same manner, that is with a sensation of constriction of the toes (usually the great toe) of the right foot, then a contraction of the toes and foot, gradually involving the rest of the leg. During the first three years he usually lost consciousness, but after that

this symptom became more and more infrequent, and for two years before we saw him it had not occurred at all. At first the convulsions were confined almost entirely to the leg, and it was only during the last years that the right arm had become affected. After each convulsion, even at the beginning of his illness, the right leg was weak and numb for a longer or shorter period. At first this weakness was only of an evanescent character and quite completely recovered from, but for about two years before we first saw him the right leg had gradually become permanently weak and the numbness constant. His general health was good; he did not complain of headache, sick stomach, or difficulty of sight.

The examination on August 26, 1896, showed the patient to be a somewhat spare, but fairly well-developed man; his intelligence was good, his speech was rather thick and guttural, but showed no actual defect; there was no aphasia. The cranial nerves performed their functions normally; there was in particular no noticeable weakness of the right side of the face. The right arm was fairly strong, although somewhat weaker than the left, which was normal. The walk was typically hemiplegic, the weakness being of the right leg. The movements of the right ankle were almost completely lost, as were those of the toes; whereas those of the knee and hip were retained, although they too showed great loss of power. The left leg was normal. The right leg was smaller than the left, the difference of measurements of the thighs being about three cm. The deep reflexes were exaggerated on the right side, and were normal on the left. There was, however, no well-marked clonus. No abnormality was discovered in the sensory condition of touch, pain and temperature. An electrical examination showed that the muscles of the right leg required a somewhat stronger faradic or galvanic current to cause them to contract than did those of the left side. An ophthalmoscopic examination, made by Dr. Theobald, showed that there was well-marked hyperæmia of the optic discs (no neuritis). A low grade of choroido-retinitis existed, "such as occurs in consequence of eye-strain, and the hyperæmia of the disc, I take it, is only part of this condition."

The patient was seen from time to time, and on September 29 he reported that on the morning of that day he had had a convulsion, which affected particularly the arm, but also had involved the face, the latter for the first time. At the time of his visit to me the right arm was powerless. He entered the hospital on October 7.

The examination, after his entrance to the hospital, differed from that given above, in that it revealed a slight weakness of the right side of the face. The right arm had regained a certain amount of power, but was

still very weak. It was also discovered that the right arm was smaller than the left by nearly four cm. in the measurements of the upper arm, and that the right foot was colder to the touch than the left. A careful examination of the sensory functions showed that the condition of touch, pain, heat and cold was normal throughout the body, except on the anterior aspect of the right leg below the knee. Between the knee and ankle the patient was unable to distinguish accurately between hot and cold stimuli, and on the foot, heat and cold produced no sensation. The muscular sense was completely lost in the right arm and leg.

The head having been shaved, three lineal scars were detected above the occipital protuberance, the longest, 4 cm., and the smallest, 1 cm. in length. The scalp was freely movable in this region, as it was everywhere over the head. Dr. Camac * discovered by auscultatory percussion that there was an area on the left side of the mid-parietal region in which the note was of a lower pitch than that over the rest of the head. It was also noted that while the veins over the left temporal region were very prominent, those over the right were not to be seen.

I had informed the patient before he entered the hospital that it was probable that there was some growth which was gradually involving the brain, but that it was impossible to determine its precise nature. The supposition that it was some chronic, syphilitic meningeal growth was entertained, and, in spite of the fact of his having denied any infection, he was put on increasing doses of iodide of potassium. After the arm had become paralysed, and the convulsions begun to involve the face, the advisability of an exploratory operation was suggested to him. A week after his entrance to the hospital he was transferred to the surgical ward, the operation being performed on October 23, 1896. Previous to the operation he had two or three convulsions of the usual character; in one of these, however, he thought that the left side of the face was going to be affected.

The operation was performed by Dr. Bloodgood, who has kindly allowed me to use his notes. The upper half of the fissure of Rolando was exposed, the object being to examine the centres both of the arm and of the leg. The surface of the brain cortex appeared somewhat different from normal; the sulci were less distinct, and the surface of the convolutions presented a finely granular appearance. The difference, however, was not great, and neither Dr. Halsted nor I considered it remarkable. I attempted to stimulate the cortex with a faradic current, but was unable to evoke any muscular contraction, even with a very strong current. No

* I am indebted to Dr. Camac's careful notes for the description of the patient's condition upon entrance to the wards of the Hospital.

gross lesion having been found, the wound was closed, and the patient returned to the ward. Upon recovering from the ether it was noted that there was complete paralysis of the right arm and almost complete of the right leg. The face was not paralyzed. On the following day the patient's mind was clear, he spoke normally and complained of no headache. He did very well for four days, his pulse falling from 136 to 92 and his temperature from 101° to about 99° F. In the early morning of October 28 he began to complain of severe pain in the head. He became dull and somewhat delirious, refused nourishment and objected very much to being disturbed. On the 29th it was noticed that he had difficulty in chewing and swallowing, and on the 31st he was hardly able to speak. On November 1 he was better, more rational and talked with less difficulty. This improvement lasted for two days, but on November 3 the pain in the head, which had somewhat lessened, returned with its old severity, he became unconscious and died on the following morning. The temperature and pulse were normal for several days before his death; in the last twenty-four hours the pulse fell from 76 to 58 per minute, and an hour before he died it was 68. The degree of paralysis varied from day to day, but no careful examination was possible on account of the condition of the patient.

After the patient's death the body was removed to his home, where an autopsy, which was confined to the head, was made by Dr. Livingood, on the evening of the same day.

The clinical aspects of the case justify a few remarks. An accurate diagnosis during life was difficult, perhaps even impossible, and was at least not made. The patient presented himself with the history of having for six years been subject to convulsions, beginning in the right foot, and stated that for the last two years this foot and leg had become weak. The examination in the dispensary, besides weakness and atrophy of the right leg with exaggerated deep reflexes, revealed nothing of importance.

That there was some irritative lesion of the left motor region was evident, and the fact that the epileptic attacks preceded any permanent paralysis by four years seemed to indicate that it was the cortex rather than the subcortical region which was primarily involved. The absence of choked discs, headache and vomiting, and the long duration of the symptoms made the presence of a growth in the brain itself appear improbable. The surmise that there was a chronic

meningeal process which had involved the motor cortex secondarily was believed to agree more nearly with the symptoms. That the lesion might be of syphilitic origin was considered, and the appropriate treatment given. The occurrence of paralysis of the right arm and the beginning involvement of the face made an exploratory operation seem advisable. The discovery post-mortem of a neuro-glioma, situated for the most part in the white matter beneath the motor cortex, which apparently had arisen in this locality, showed, however, that our assumption was incorrect. It is, to say the least, remarkable that such a growth should have existed in the brain for so long a time without causing the characteristic general symptoms.

The occurrence of Jacksonian epilepsy, followed at a later time by paralysis of the affected limb, in connection with a subcortical tumor, had been observed more than once before.* That the motor cortex would be inexcitable at the time of the operation is what one would expect from the lesion found. This lesion also explains the loss of the muscular sense in the arm and leg, and the disturbance of the temperature sense in the lower part of the leg, in accordance with our present conception that the central convolutions are the centre both for sensation and motion.

The altered percussion note over the region of the tumor, which was discovered by Dr. Camac on the day before the operation, is particularly interesting and emphasizes the importance of this sign as a help in the local diagnosis.†

The muscular atrophy of the right arm and leg is of particular

* Oppenheim, Die Geschwülste des Gehirns, in Nothnagel's Spec. Path. u. Ther. ix, I, 3, p. 73, Wien, 1896.

† See Bruns in Eulenburg's *Real-Encyclopädie*, viii, 658, 1895.

This sign was present in two cases of tumor of the frontal lobe which were operated on for me. In the first case it was pointed out by Dr. Allen Starr [*Jour. of Nerv. and Mental Dis.*, Oct., 1896, p. 660], and in the second case, in which it was very evident, the frontal bone, at the time of the operation, was found to have been reduced to the thinness of paper. In a third case I believed the sign to be present, but at the autopsy no tumor or other condition was found to explain it. From my own experience I should say that the sign is one about which it is easy to be deceived, and upon which reliance should be placed only when the difference in the percussion note is well marked and constant.

interest. It was of the nature of a general diffuse atrophy, involving all the muscles of the limbs; these muscles responded normally to the electric currents, although they required a stronger current to produce a response than is usual. That muscular atrophy does at times follow a lesion of the brain has been known for many years, but only recently has particular attention been called to the condition. Steiner* collected and analyzed the published cases and reported a personal observation, making in all eighteen cases. Since that time a considerable number of additional cases have been published. The subject is a most interesting one, and has given rise, in the attempt to explain the occasional occurrence of muscular atrophy associated with lesions of the brain, to several theories. These differ widely from each other, and involving, as they do, important physiological problems, their detailed consideration is beyond the scope of the present article. From a study of the cases it appears that muscular atrophy may follow any sort of lesion, situated anywhere in or near the motor path, within the brain. The lesion may be such as to destroy the continuity of the motor neurones, and thus be followed by secondary degeneration of the pyramidal tract; or there may be no evidence of such degeneration, and, indeed, the motor paralysis itself may be a very inconspicuous symptom.

In our case, Dr. Hamilton was unable to discover, even by the use of the newer methods, any secondary degeneration, and it is remarkable that in two of the cases in Steiner's list (Case VII, Roth and Muratow, and Case IX, Quincke), in which muscular atrophy followed the growth of a tumor in the motor region, the pyramidal tracts were normal. Finley† has very lately reported two instances of tumors of the brain in which secondary degeneration was absent. In one the growth was of the optic thalamus, and in the other it was of the crus cerebri.

* *Deutsche Zeitschr. f. Nervenheilk.*, lii (1893), 280.

† *Montreal Medical Jour.*, xxv (1896), 208.

Part II.

The Pathological Study.

By ALICE HAMILTON.

The histological study was limited to the cerebrum and medulla. It may be regretted that the spinal cord was not available also; but it is believed that the conclusions arrived at regarding the absence of secondary degenerations in the pyramidal tracts would not have been altered by an examination of the cord in its lower levels.

The seat of the operation was immediately around the Rolandic fissure. The meninges covering the affected area were dark in color and hæmorrhagic. In the formalin-hardened hemisphere the region of more or less involved tissue is composed of the ascending frontal, the ascending parietal and the third frontal convolutions. Externally the involved area occupies a smaller volume than the parts beneath the cortex. The immediate site of disease is indicated by a softened and depressed focus 2 to 3 cm. in width, from the centre of which projects, in the form of a hernial protrusion, a shaggy mass of brain tissue as large as a walnut, covered by membranes. The posterior superior tip of the third frontal convolution is impinged upon by the hernia and is pressed forwards, giving rise to a crescentic excavation 1.5 cm. long. A part of the hernia is derived from this convolution, which is softened. The cortical upper surface of the ascending frontal convolution is free from any gross lesion; on the other hand the surface within the Rolandic fissure projects forward, forming a distinct tumor-like bulging, which extends quite to the base of the convolution. On separating the ascending frontal from the ascending parietal convolution, the central portion, corresponding to the softened area, is occupied by a pulpy mass, which is found to be continuous with the surface of the latter. The cortical surface of this convolution in its entire length, except for the slight bulging in the lower part, appears normal; but on its Rolandic surface, in its lower half, there is a tumor-like projection, the cortical surface of which is firm, the interior softened.

A close study of the distribution of the diseased area made from time to time during the progress of the work shows that, while the foregoing description is accurate as far as the appearances seen on the surface are concerned, a section of the hemisphere and the gradual removal of more and more tissue from the affected convolutions made evident a tumor

invasion through the bases of these convolutions, extending into the centrum semi-ovale backward so as to involve the beginning of the optic radiation and forward within the area of the termination of the white matter in the frontal region. Below, it extends into the corpus callosum in its median and anterior portions, the posterior third seeming to escape. The softening extends to but does not enter the thalamus. There can be no doubt now that the softening is not post-operative, but due to the tumor.

From all parts of the tumor small portions were taken and transferred, some to alcohols of increasing concentration, others to Müller's fluid. The specimens hardened in alcohol were stained with Upson's carmine, hæmatoxylin and eosin, Van Gieson's picric-acid-fuchsin, Mallory's phospho-molybdic-acid hæmatoxylin and Nissl's methylene blue, while those hardened in Müller's fluid were treated by Marchi's and by Weigert's (medullated nerve) methods. Other specimens were treated by Golgi's silver chromate method and, finally, the attempt was made to demonstrate the neuroglia by Mallory's method, but without success, probably because the tissue was not fresh enough or handled exactly in the prescribed manner.

The results obtained by these different methods when combined give a very clear picture of the elements composing the tumor. Those specimens which were stained by Mallory's phospho-molybdic stain were perhaps the most valuable, as this stain shows with great clearness the cell processes and the outlines of those cells which have invisible cell membranes, and whose protoplasm is therefore difficult to see unless treated by so intense a staining agent as this.

A great variety of cells is seen, some typical examples of which are shown in the figures of Plates L-LIII. It would, however, have been necessary to draw a very great number of cells in order to show all the types, so great is their diversity. It would therefore be very difficult to classify them according to shape, but when one examines the character of the nuclei and of the protoplasm, the cells seem to fall into two quite distinct classes. Of these one is characterized by an invisible cell-membrane, protoplasm that stains faintly and processes which, if present, are exceedingly delicate. The nuclei in these cells are often slightly stained, but have a dark periphery and exhibit threads and granules of chromatin. The other class is distinguished by granular protoplasm, distinct outline, thicker processes, and usually a deeply stained nucleus. These two classes are not absolutely distinct, they merge into each other in some cases, but on the whole the two types are well separated. One may therefore say that all the cells with granular protoplasm are furnished with

distinct processes. Besides these, many normal neuroglia cells are present in addition to those which vary somewhat from the normal type.

Spindle cells are found in great numbers, some with single small nuclei, but a far greater number with two or three nuclei or with one large irregular nucleus. These cells are quite large and are furnished with remarkably long processes at one or both ends, or with a brush of many fibres, when they correspond with the so-called brush cells (*Pinselzellen*). The processes are seen sometimes to branch after leaving the cell (Plate L, *A*, *B*, *C*).

Of the same class, *i. e.*, with lightly staining protoplasm and dark nuclear membrane, are certain very large cells, usually more or less triangular in shape, provided with projecting nuclei numbering sometimes as many as eight in a single cell. These give rise to appearances very like that of giant cells in certain sarcomata (Plate L, *F*, *H*). At times the nuclei are grouped in a wreath around the periphery of the cell. Other large cells have very little protoplasm and look often like masses of naked nuclei grouped in clumps, some of them taking the stain deeply, and others only slightly (Plate LII, Fig. 1). Again, a clear vacuole may appear within a cell, pushing some of the nuclei to the edge of the cell and altering their shape. Even with Mallory's phospho-molybdic acid stain it is impossible to demonstrate with absolute certainty the presence of protoplasmic processes for these large cells; and in specimens stained by other methods there is absolutely no suggestion of such extensions. A notable absence is the large neuroglia cell with numerous radiating processes, the spider-cell (*Spinnenzelle*), which is nowhere to be seen.

Among the spindle cells described above are some which have granular protoplasm, like that of ganglion cells, and thick and coarse processes. The same is true of certain of the large triangular cells. But by far the greater number of the cells whose protoplasm is of this character impress one as being atypical ganglion cells. They assume various shapes and sizes and may be multinucleated. In no case, however, was pigment found in them and their nuclei are devoid of nucleoli. Yet they have the general character of ganglion cells. Still other cells look like neuroblasts, except that their protoplasm is more abundant and their nuclei stain more deeply and uniformly than is the case with the normal embryonic cells. These cells have distinct processes often thick and long and branching at a distance from the cell-body.

The blood-vessels are not especially numerous in any part of the tumor. In some parts they appear normal, but usually the walls are thickened by spindle cells (Plate LII, Fig. 1). These cells differ in no way from the

spindle cells throughout the tumor, they have processes at one or at both ends and usually lie close to the vessel wall with the long axis parallel to the course of the vessel. In some places these accumulations of spindle cells are very thick and in carmine specimens they stand out as deeply stained masses, always arranged evenly around the vessels and never under any circumstances pushing their way into the surrounding tissue.

Medullated nerve fibres are found in all parts of the tumor, also many devoid of the medullated sheath (Plate LIII, Fig. 4).

As the tumor, beginning with those sections nearest the cortex and going in to the centre of the tumor, is examined, the elements described above are found in varying abundance and arrangement (Plate LI). In the more superficial sections the ground substance is the usual fibrillar neuroglia network. Here are found the round cells with small nuclei, the spindle and brush cells whose processes begin, as one penetrates further down, to give to the ground substance a different appearance. Sections which approach the centre show the large multinucleated cells with clear, light protoplasm or with almost no cell body at all. The first cells of the second class to appear are the irregular spindle cells and those resembling neuroblasts; and finally the centre of the tumor is composed of irregular ganglion cells whose processes form a thick felt-work surrounding the cells. Comparatively few of the cells with clear protoplasm are found here except around the blood-vessels.

This, however, is only the general arrangement of the tumor; there are deviations from this type in some places. For instance, there is found in one place just below the cortex a nodule about the size of a hazel-nut, which macroscopically seems to be quite circumscribed and distinct from the rest of the tumor. Under the microscope this nodule is found to be composed of cells of the smaller type, round or slightly oval, usually with but a single nucleus, and very much more closely packed together than elsewhere in the tumor (Plate LII, Fig. 2, *B*). The appearance presented by this mass is quite different from that of the surrounding tumor tissue, especially in specimens stained with carmine or with hæmatoxylin, for in these the cells seem entirely devoid of processes. Only by using Mallory's phospho-molybdate stain could the presence of numerous very delicate processes be demonstrated. Near the edge of this mass the cells grow larger and more spindle-shaped (Plate LII, Fig. 2, *A*), but are still very densely packed together, and there is a sharp line of demarkation between them and the loose, large-celled tissue surrounding the mass (Plate LII, Fig. 1).

The tumor is for the greater part of its extent confined to the white matter, involving the cortex only in a few places. But the latter in this

situation, even when uninvaded by the new growth, is nowhere entirely normal. There is a decided increase in the number of small round cells; in one place karyokinetic figures were seen near the surface of the cortex within what were apparently neuroglia cells. In other places scattered tumor cells are found extending even up to the outer surface and, in one spot, a mass of closely packed round cells fills all the extent of the cortex. These are unquestionably tumor cells, not polymorphonuclear leucocytes. The latter are found penetrating here and there among the cells of the cortex and the upper part of the new growth, but they are few in number and always lie singly. There is therefore no evidence of any considerable inflammatory reaction, not even along the cortical surface and in the membranes.

In the lower layers of the cortex, near the new growth, there begin to appear round and spindle cells and giant cells. The normal pyramidal cells are still present, but there is no evidence of proliferation on their part. Here also, in the large-celled layer of the cortex, are found certain remarkable cells which look like enormous multipolar cells of the anterior horns (Plate LIII, Fig. 3).* They are normal in shape and structure, furnished with processes and with a single nucleus containing a distinct nucleolus. They are much larger than any pyramidal cells normally found in the cortex and they do not belong to the type of pyramidal cells, but resemble the largest of the multipolar cells of the cord, some even exceeding the latter in size.

Sections passing through both cortex and tumor, which have been stained by Weigert's hæmatoxylin method, show the medullated fibres passing down from the cortex to enter the tumor and, some of them at least, to pursue their course and emerge below to pass on to the internal capsule (Plate LIII, Fig. 4). The fibres seem to be simply pushed apart by the tumor cells, or sometimes when these cells lie closely packed together, as in the nodule described above, most of the fibres are forced to each side, curving around the mass to meet below. Examination under the higher powers brings to light many fibres which seem to lose their myelin sheaths on entering the tumor. The denser parts of the tumor especially show many such non-medullated fibres. That not many nerve fibres have undergone degeneration as a result of their passage through the new growth is shown by specimens from this same region stained by Marchi's method. Here can be found scattered single degenerated fibres, but never large tracts of such fibres.

Beginning with the internal capsule and passing down through crus,

* These cells resemble the "Betz cells" of the paracentral lobule, and doubtless are homologous with them.

pons and medulla as far as the decussation of the pyramids, serial sections were made and stained by Weigert's method. These gave very surprising results, for nowhere could any degeneration of the fibres of the motor tracts be discovered. Even a part of the crus prepared by Marchi's method failed to give any decided result, the isolated fibres, which gave the reaction, being too few and scattered to be of any significance.

In considering the nature of this tumor and the class of neoplasms to which it belongs, one finds that the question resolves itself into that of neuro-glioma or glio-sarcoma. A study of the literature on the pathology of brain tumors shows that either one of these two positions could be supported by many and eminent authorities. Perhaps it will be well at this point to review briefly what have been and are the opinions held by various authors on this subject.

The literature on glioma* begins, of course, with the appearance of Virchow's "Die krankhaften Geschwülste." Virchow separates gliomata from sarcomata and makes the separation depend on the character of the cells. In glioma the cells are of the type of neuroglia cells, they have always processes, which form an essential part of the ground-substance, and nerve cells and nerve fibres are always absent. The cells are proportionally few in number, the ground-substance is the more prominent part. Sarcoma, on the other hand, consists, of course, of polymorphous cells devoid of processes, and very numerous, while the granular cement substance is relatively scanty.

This classification was first objected to by Klebs, who maintains that nerve cells form a large part of some gliomata and are the only constituents of others. Following his well-known theory that in a new growth all parts of the tissue proliferate, Klebs asserts that there are tumors in which all nervous elements including axones are represented, yet which may resolve into gliomata. The character of the cell therefore, according to this view, does not determine the nature of the tumor.

Golgi, on the other hand, follows Virchow in basing the distinction between glioma and sarcoma on the characters of the cells, laying special stress on the cell processes. According to him a glioma must consist essentially of cells with radiating fibres, *i. e.*, spider cells. Jastrowitz describes as the typical elements, cells with scanty protoplasm, large clear nuclei and numerous processes. Hektoen follows Virchow and Golgi in

* References to literature are at the end of this article.

considering the presence of fibres the most important distinguishing point. He calls the tumor he reports a glio-sarcoma because it consists of cells furnished with processes similar to glia-cells, and also of round cells which seemed to be devoid of processes.

Practically all of the earlier writers accept this classification, though some lay more emphasis on the relative number of cells in the tumor than on their character. The results are therefore very confusing, for two cases which seem by the descriptions essentially alike, are classed, the one among the gliomata, the other among the sarcomata, according as they seemed more or less rich in cells. The conception of a glio-sarcoma is even more vague, the term being usually applied to a glioma composed of two or more different kinds of cells. In Lemcke's very exhaustive article 97 cases are collected from the literature up to 1881, 17 of which are reported as glio-sarcoma, 80 as pure glioma, but the description of the microscopic appearances is essentially the same in all and it is impossible to see why certain ones were supposed to belong to the mixed type and others not.

However, not all of the earlier authors accept the dictum that a tumor composed of cells with processes must be a glioma. Marchi considers the number of cells more important and classes among the sarcomata a tumor consisting of cells resembling neuroblasts and spindle cells with many fibres. The cells in this case were very numerous and the cement substance slight in amount. Fenoglio does the same. Indeed his typical sarcoma cell is exactly like Jastrowitz's typical glioma cell. Even down to the present day there are authors who still base their classification of brain tumors upon the character of the constituent cells, as Birch-Hirschfeld in his "Allgemeine Pathologie," 1892. A case described by Galavielle and Villard in 1895 is unhesitatingly denominated sarcoma because of the richness and variety of its cellular elements.

In the case of the earlier authors it is easy to understand why this should be so. The neuroglia was at that time looked upon as one of the forms of connective tissue, mesoblastic in origin, and a glioma therefore belonged to the connective tissue tumors, differing from sarcoma only in having its starting place in the modified connective tissue of the nervous system, and therefore resembling neuroglia, but capable of passing gradually over into pure sarcoma. This is very clearly expressed by Gowers, who puts glioma and sarcoma as subdivisions of the general class, mesoblastic new growths. Fleischl goes still further and proposes abandoning the term glioma, since it simply means a sarcoma which had its origin in the neuroglia and which, elsewhere in the body, would be called fibro- or myxo-sarcoma. Even a tumor consisting entirely of elements resem-

bling ganglion cells he calls a sarcoma originating from the pyramidal cells of the cortex.

After the establishment of the epiblastic origin of the neuroglia the distinction between these two classes of growths could be much more distinctly made, as also the close connection between glioma and neuroglioma. The only mesoblastic tissue in the brain being now known to be in the sheaths of the blood-vessels, it followed that any connective tissue tumor in the brain must originate either in the brain membranes or in the vessel walls. Thus Ziegler in his last edition limits the term glio-sarcoma to gliomata in which the connective-tissue cells of the vessel walls also undergo increase and form an integral part of the tumor. Stroebe and Oppenheim give the same definition of glio-sarcoma. Von Lenhossék inclines to the belief that all new growths occurring in the central nervous system are gliomata or neuro-gliomata. He admits the possibility of a mixed growth in which the cells of the vessel walls participate and would call such a tumor glio-sarcoma, but he refuses to accept as such any of the cases which have up to this time been described.

It seems therefore most logical to restrict the term glio-sarcoma to a tumor of undoubted mixed origin, yet the difficulty of applying this rule is great. Almost all authors speak of a thickening of the vessel walls as being present in gliomata; the cells lying near the vessels become more spindle-shaped and group themselves around the vessel in several layers. In most cases these cells do not differ in any way from the other spindle cells occurring in the tumor, but the latter are too evenly scattered to be regarded as having any connection with those around the vessel. In other gliomata the cells are all round with the exception of those in the vessel walls, and this has in some cases led the author to consider the growth a mixed one. It would seem, however, that as long as these cells are grouped evenly around the vessels and show no tendency to form masses pushing out into the surrounding tissue, as long as they do not, as Ziegler says, form an integral part of the tumor, they should be regarded simply as a proliferation of the cells of the vascular wall, due perhaps to the irritation of the neighboring tumor.

Still another view as to the part played by the blood-vessels is emphasized by De Beauclair, who describes three cases of "sarcoma" of the brain and gives as the most distinguishing feature of these growths their naked vessels, which lie directly in contact with the tumor cells, being without any limiting membrane. As this is characteristic of the vessels in sarcoma elsewhere in the body, the point is not an unimportant one. Buchholz wishes to establish as an important characteristic of glio-sarcoma the fact that many glia cells near the blood-vessels are furnished

with a long process which passes to the vessel wall and ends there in a triangular swelling. This peculiarity I do not find mentioned by any one else.

On the whole the term glio-sarcoma has been, even up to the present time, very loosely applied, and while accepting the definition of the later writers as probably correct, one is inclined, after studying the literature, to agree with von Lenhossék that no undoubted case of glio-sarcoma has yet been reported. Indeed, in the absence of drawings it would be hard to prove such a case in the literature.

Admitting the purely ectoblastic origin of all the cells in a neuro-glioma, there is still a difference of opinion as to the ganglion cells in such a growth, whether they are to be regarded as arising from the pre-existing ganglion cells or from the glia cells. Klebs holds that either may be true, that a new growth may have its starting point in the neuroglia and go on to the production of ganglion cells, or that it may start in the pyramidal cells of the cortex and also produce both neuroglia and ganglion cells. Golgi doubts the possibility of any proliferation on the part of the real nerve elements and prefers to regard those present in the tumor as pre-existing cells modified by the pathological process. This, however, would not apply to those cases in which the ganglion cells are enormously increased in number and found in situations where no ganglion cells exist normally, as in several of the cases reported by Klebs, in Osler's case, in the present case, and in others.

Both Klebs and Fleischl claim that they find ganglion cells in the act of unequal division, this division taking place simply by cleavage of the protoplasm, the nucleus seeming to take no active part in it. Their drawings show large irregularly shaped, multinucleated cells with one or two furrows or clefts in the protoplasm dividing them into unequal parts, one of which may be without a nucleus. In some cases cleavage seems to be not yet complete. Therefore these two authors consider the ganglion cells composing a neuro-glioma to be produced by the splitting of pre-existing pyramidal cells. This question I will return to later on; although appearances identical with those just described are found in my case (Plate L, *D*), they are capable, I think, of another interpretation.

The possibility of a proliferation of the pyramidal cell of the cortex has indeed been lately held by Vitzou and by Tedeschi. These two observers have, independently of each other, arrived at the conclusion that after extirpation of part of the cortex the nerve elements may proliferate. The great mass of opinion, however, is still against this position, and as regards the ganglion cells in a neuro-glioma the view most generally held is that all the elements in such a growth are derived from

indifferent embryonic cells, which may develop into neuroglia cells, or go on to the production of ganglion cells, or both. This view is held by Stroebe, by Raymond, by Tedeschi, by Fenoglio, by von Lenhossék, and by Lesage and Legrand. These two last-named authors found in a congenital tumor in a new-born child cells which represented all stages of development, none of them varying in any way from the types of normal embryonic cells. Hartdegen also finds only normal embryonic cells, though in abnormal situations. His case was also one of congenital tumor. Stroebe found, besides typical embryonic cells, many abnormal elements in several of his cases; irregular ganglion cells and multinucleated giant cells, but he considers them all to have originated in the neuroglia.

Raymond claims that he could in his case trace the development of the ganglion cells, which filled the centre of the tumor, from their earliest stage to complete development. He begins with the small round cell, which lies in the pericellular space around a ganglion cell and which, though in appearance just like a neuroglia cell, is capable of replacing the ganglion cell, if the latter perishes. This cell proliferates and in the next stage the space formerly occupied by the ganglion cell is found filled with small round cells. These take on the character of neuroblasts, often becoming multinucleated and sending out processes. The protoplasm increases and they reach the stage of atypical ganglion cells. The first stages are found near the edge of the tumor, the last in the centre. The drawings which accompany his article show examples of all these different stages, but hardly establish his first point, the starting of the process in the small cell near the ganglion cell. Nor can I find any authority for assuming the existence of such an *Ersatzzelle*. A small round cell is frequently observed in the pericellular space of a nerve cell, but I cannot find that it is considered by anatomists as a cell capable of developing into a true nerve element.

Renaut lays more stress upon the reversion of the cells in a neuroglioma to the type of those in the lower animals, and draws his comparisons from ganglion cells of the lower invertebrates, rather than from human embryonic cells. Fenoglio is unwilling to regard these so-called ganglion cells as in any sense true nerve elements, while Klebs and Fleischl consider them really nerve cells, and Renaut insists that he has even found some of the glia cells sending out true axis cylinders, which entitles them also to be regarded as nerve elements. This last seems somewhat doubtful, especially when one considers that he bases his assertion on appearances found in specimens stained only by the usual methods, hæmatoxylin and carmine. Osler, who found many ganglion cells in his case, does not consider them true nerve elements.

Probably a distinction should be made here between growths which occur in adult life and those which are congenital; the latter, being caused by misplacement or irregular development during fœtal life, would be composed of cells more like the normal.

To sum up then, briefly, the conclusions that may be drawn from the literature on this subject, it will be seen that the older view, which held that the nature of a new growth within the brain depended on the number and character of the cells composing it, has been abandoned. All new growths in the brain, being epiblastic in origin, are to be regarded as gliomata, unless they are proved to have as starting-points the membranes or the walls of the blood-vessels. A mere thickening of the vessel wall is not to be so interpreted. The term glio-sarcoma is to be limited to those tumors which show pathological increase both of the neuroglia on one side and of the connective tissue belonging to the vessels on the other, the latter being considerable enough to form an integral part of the tumor. Neuro-gliomata have the same origin as other gliomata, there being probably no proliferation on the part of the pre-existing ganglion cells, but all the cells of the tumor representing earlier or later stages of development of the original indifferent embryonic cells in which the process started. These cells are often far from any normal type and may not resemble either neuroglia cells or nerve cells. Such atypical ganglion cells are not true nerve cells and are probably not furnished with genuine axis cylinders.

Returning now to the tumor described above and considering it in the light of these conclusions, one cannot do otherwise than class it among the neuro-gliomata. Were one to judge simply by the characters of certain of the cells or by the richness in cellular elements, one would undoubtedly pronounce it a sarcoma. The great variety of cells, the presence of giant cells, some even devoid of processes, the absence of typical spider cells, and the fact that the cells sometimes lie in direct contact with each other, have all been taken as grounds for such a diagnosis. Or admitting that the mass of the tumor consists of cells which are epiblastic in origin, the increase of spindle cells in the walls of the vessels might lead to its being considered a

mixed growth, a glio-sarcoma. As we have seen, however, this increase is too slight to be considered sarcomatous in character, and as the mixed nature of the tumor cannot be sustained, it must be considered as belonging to the neuro-gliomata.

As to the question whether the irregular ganglion cells composing a large part of the tumor arise from pre-existing ganglion cells or merely represent the latest stage of development of the tumor cells, the appearances in this case seem to bear out the latter view. It must be admitted that ganglion cells are to be found which present appearances interpreted by Klebs and Fleischl as evidences of cell division, some of which are shown in the plates. But it seems far simpler and less presumptuous to suppose that these are cells in close apposition, whose shape has been altered by pressure, or, if there really is a beginning and still incomplete division, that it is of the nature of a degenerative change and not a true cell division. There is, besides, no evidence of the multiplication of pyramidal cells in the cortex, and the ganglion cells of the tumor are found, not in the cortex, but in the centre of the mass, in the white matter.

Karyokinetic figures were found only in the glia cells. The cells on the outer edge seem to represent earlier stages of development, those in the centre, later. Yet they do not represent merely different stages in the development of adult ganglion and neuroglia cells from indifferent embryonic elements. Many, indeed almost all, of the cells are atypical, some of them hardly resembling at all their prototypes, and are recognized as belonging with them only because transition forms between them and normal ganglion or neuroglia cells can be found. The cells first described, which have light, clear protoplasm, nuclei with dark periphery and delicate processes, seem to be products of cells which took the character of neuroglia, while those with granular protoplasm, dark, uniformly stained nuclei, and thick processes seem to belong to the types of nerve cells. That the latter are not true nerve elements seems proved not only by their deviating from the normal type in shape and in the structure of their nuclei, but also by the fact that Nissl granules cannot be demonstrated. They also failed to react to the Golgi method, but I am inclined to attribute

this to the imperfect preservation of the tissue, although Tedeschi and Osler, almost the only ones who have used this method in the examination of gliomata, state that the ganglion cells in their cases did not take the silver chromate. Osler, indeed, considered this an additional proof that they were not true nerve elements.

As regards the absence of degeneration of the motor tracts below the centrum semi-ovale, the cause is made clear by examining those sections of the tumor which were stained by Weigert's method, and where the fibres can be seen entering the mass, some losing their medullary sheaths, others persisting unchanged and emerging below. This appearance is very similar to that found in the so-called insular sclerosis (Charcot) of the cord, when, as is well known, the naked axis cylinders are found pursuing their course through the sclerotic patches, and the tract below shows no secondary degeneration. It is even conceivable that those fibres which lost their myelin sheaths in their passage through the tumor may have regained them on emerging. This interruption in the myelin sheath of a nerve is seen in the nerves in the mesentery of some of the lower vertebrates, and in the sensory nerves in man in their passage through a Pacinian corpuscle (Schiefferdecker). Also, as von Recklinghausen first pointed out, in neuro-fibromata of the skin the nerves take their course through the fibrous tissue partly as medullated fibres, partly as naked axis cylinders, and in all probability the latter were formerly also furnished with myelin sheaths.

I am glad to take this opportunity to express my gratitude to Dr. Flexner for his kind direction and assistance throughout this investigation; and also to Dr. Barker for many helpful suggestions.

DESCRIPTION OF PLATES L-LIII.

Plate L.

Isolated tumor cells. *A*, *B*, *C*, various forms of spindle cells. *D*, dividing (?) ganglion cell. *F*, *H*, non-branching giant cells. *E*, *G*, *I*, glia and neuroblastic elements. Phospho-molybdate stain.

Plate LI.

Vertical section through the affected motor convolutions. *A*, meninges; *B*, cortex showing invasion of tumor; *C*, white matter containing the main growth. Phospho-molybdate stain.

Plate LII.

Fig. 1. Typical field showing multinucleated and branching (ganglion?) cells, brush cells, etc. Same staining as previous ones.

Fig. 2. Two fields from the circumscribed nodule (page 645). *A*, spindle and branching cells; *B*, round and oval cells devoid of branches. Phosphomolybdate staining.

Plate LIII.

Fig. 3. Large multipolar (Betz) cell among pyramidal cells of the cortex and small tumor cells.

Fig. 4. Invasion of tumor cells between the medullated nerve fibres, some of which show varicosities. Weigert's myeline stain.

BIBLIOGRAPHY.

Baumann.—Inaug. Diss., Tübingen, 1887.

De Beauclair.—Inaug. Diss., Freiburg, 1891.

Birch-Hirschfeld.—Lehrb. d. allg. path. Anat., Leipzig, 1892.

Bramwell.—Intracranial Tumors, Edinburgh, 1888.

Buchholz.—*Arch. f. Psychiatrie*, xxii (1890).

Carrara.—*Giornale d. r. Accad. d. Med. d. Torino*, 1895, 270.

Ernst.—Ziegler's *Beiträge*, ii (1892).

Fenoglio.—*Gazz. delle Cliniche di Torino*, 1876.

Fleischl.—*Wiener med. Jahrbüch.*, 1872.

Francis.—*Am. Journ. Med. Sciences*, 1895.

Galavielle and Villard.—*Arch. de Neurologie*, 1895, xxx, 1.

Golgi.—*Rivista Speriment. di Freniatria*, 1875, 1.

Gowers.—Diseases of the Nervous System, 1892.

Hartdegen.—*Arch. f. Psychiatrie*, 1881.

Hektoen.—*Journ. Am. Med. Assoc.*, 1893, 20.

Jastrowitz.—*Arch. f. Psychiatrie*, iii.

Klebs.—*Prager Vierteljahresschrift*, 1877, 138.

Klebs.—Die allgemeine Pathologie, Jena, 1889.

Laveran.—*Bull. et Mém. d. Soc. Méd. des Hôp. d. Paris*, 1893.

Lemcke.—Langenbeck's *Archiv*, xxvi (1881).

Von Lenhossék.—Der feinere Bau d. Nervensystems, Berlin, 1895.

Lesage and Legrand.—*Arch. de Physiologie*, 4. Sér., ii (1888).

Marchi.—*Rivista Speriment. di Freniatria*, ix (1883).

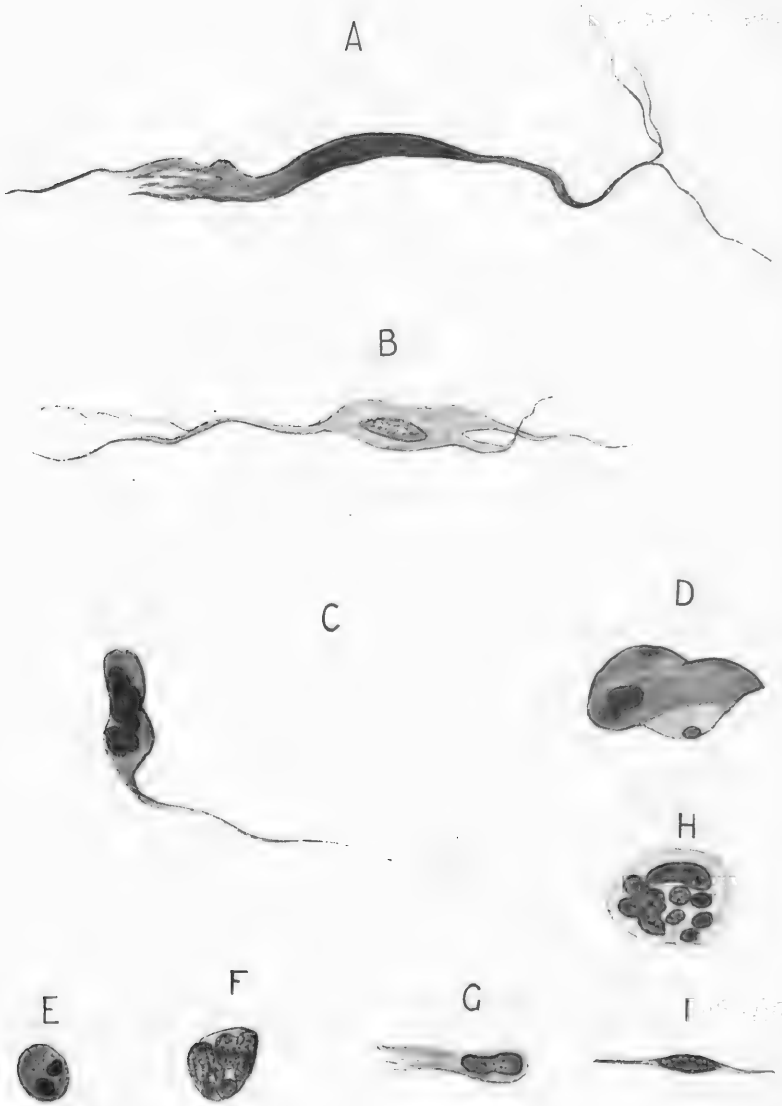
Neumann.—Virchow's *Archiv*, lxi.

Oppenheim.—*Arch. f. Psych.*, xxi (1889-90).

Oppenheim.—Die Geschwülste des Gehirns, in Nothnagel's Spec. Path. u. Ther., ix, I, 3, Wien, 1896.

Orth.—Lehrbuch d. spec. path. Anat., 1894.

Osler.—*Medical News*, 1886, 48.
Perls.—Lehrb. d. allgemeinen Pathologie, Stuttgart, 1877.
Pfeiffer.—*Deutsche Zeitschft. f. Nervenheilk.*, 1894.
Prautois and Étienne.—*Arch. de Neurol.*, 1894, 270.
Raymond.—*Arch. de Neurol.*, 1893, 26.
Renaut.—*Gaz. Méd. de Paris*, 1884.
Schiefferdecker and Kossel.—Gewebelehre d. menschlichen Körpers, Braunschweig, 1891.
Simon, Th.—Virchow's *Archiv*, lxi.
Sokoloff.—*Deutsch. Arch. f. klin. Med.*, xli (1897).
Stroebe.—Ziegler's *Beiträge*, xviii (1895).
Tedeschi.—*Rivista Speriment. di Freniatria*, 1894.
Tedeschi.—*Centralbl. f. allg. Path.*, vii (1896).
Virchow.—Die krankhaften Geschwülste, Berlin, 1863.
Vitzou.—*Arch. de Physiologie*, 5. Sér., ix (1897), 29.
Ziegler.—Lehrbuch d. path. Anat., Jena, 1895.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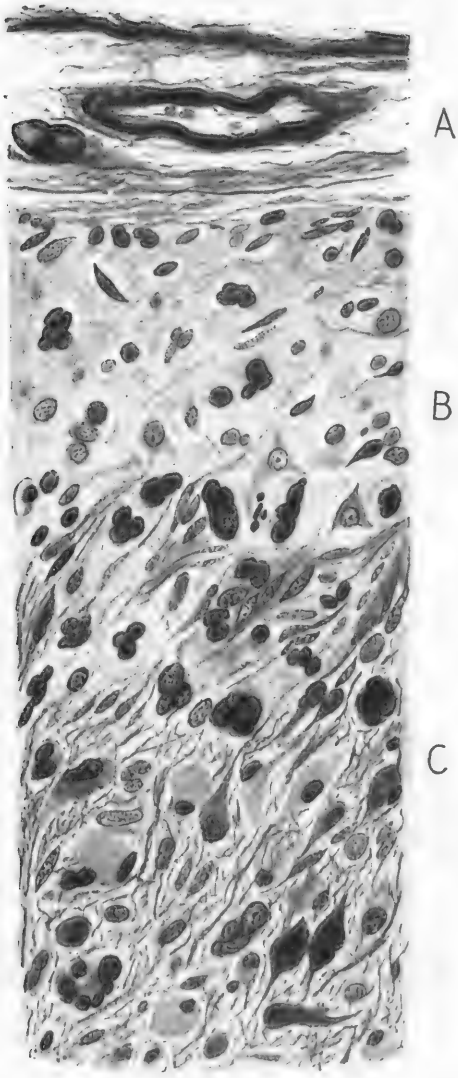
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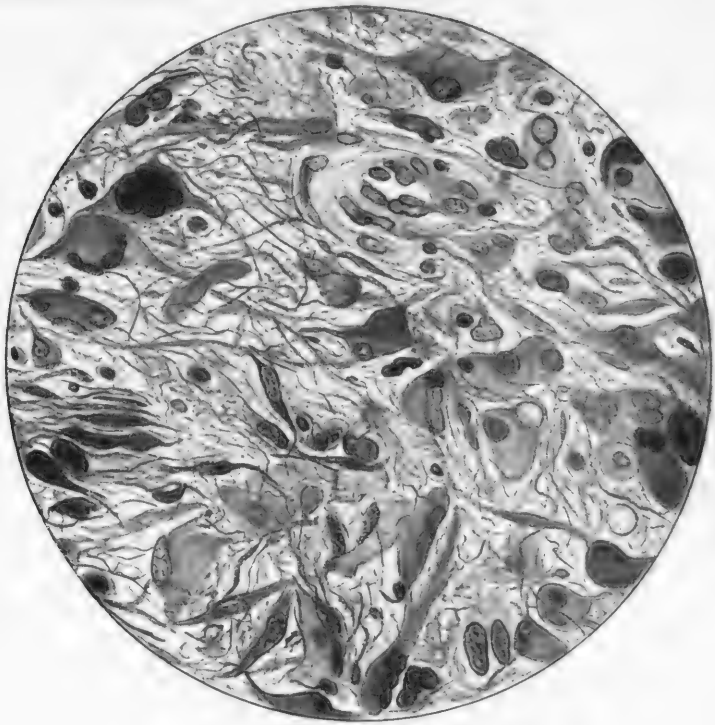


FIG. 1.

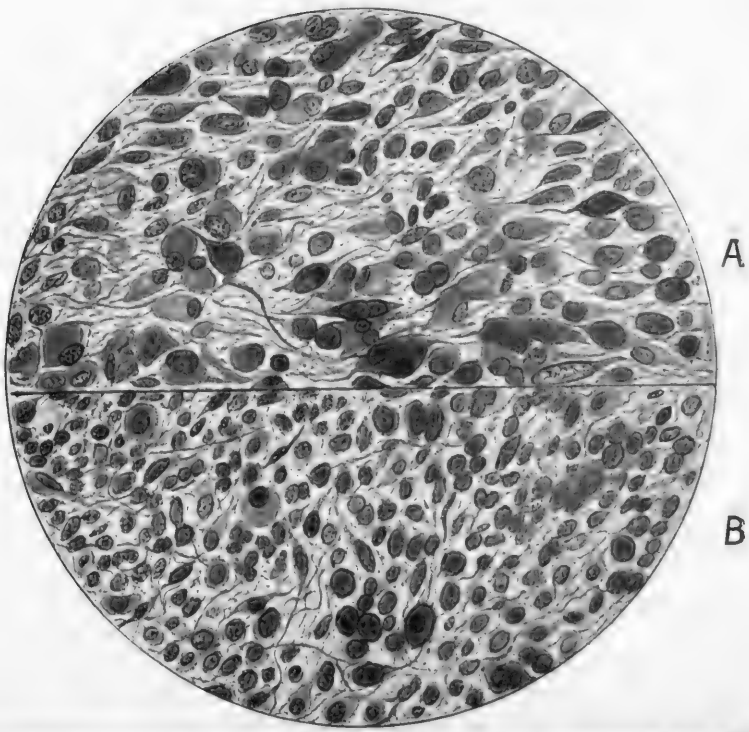


FIG. 2.

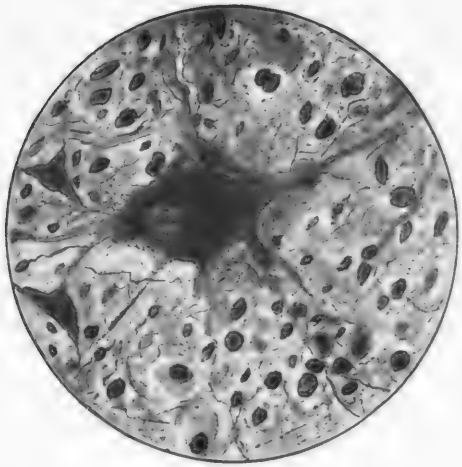


FIG. 3.

FIG. 4.

FOCAL OR INSULAR NECROSIS PRODUCED BY THE BACILLUS OF TUBERCULOSIS.

By E. R. Le COUNT, M. D., Demonstrator of Pathology, Rush Medical College, Chicago.

(*From the Pathological Laboratory of Rush Medical College.*)

Plate LIV.

Since Virchow described the cellular tubercle, it has been adopted as the anatomical basis for the lesions of tuberculosis; but the doctrine that the effect of the bacillus of tuberculosis and its products upon the tissues is invariably a specific one, having for its end the formation of a tubercle, is no longer believed. This was indicated when Baumgarten (1) defined a tubercle as the "result of proliferative and exudative changes," for the preponderance of exudative changes on the one hand or of proliferative changes on the other leads to lesions which differ morphologically and which cannot be considered as having for their main constituent the presence of tubercles. The researches of Orth (2) upon caseous pneumonia proved that in this condition the effect upon the tissues of the bacillus of tuberculosis is almost purely an inflammatory one, resulting in the formation of fibrin without the formation of tubercles.

An analogous conclusion that a multiplicity of lesions which differ morphologically one from another can occur as lesions of tuberculosis may be drawn from the experiments which have been made with dead tubercle bacilli. Although these experiments were made to demonstrate that the dead bacilli contain within themselves substances capable of instituting changes in the tissues, a most interesting feature of the results obtained was afforded in the variety of the lesions produced, and it is safe to assume that the differences that exist between the action of living and of dead tubercle bacilli upon the tissues are chiefly quantitative differences. Thus when one summarizes the results obtained by different investigators it is learned

that in the hands of Koch (3), Wyssokowicz (4), and Prudden and Hodenpyl (5), subcutaneous injection of dead tubercle bacilli produced aseptic suppuration. By Vissman (6), Straus and Gamaleia (7), Prudden and Hodenpyl, and Masur (8), through intravenous injection, tubercles were produced in the lungs, but also an interstitial pneumonia with formation of fibrous tissue and endarteritis. Abel (9), by the injection of dead tubercle bacilli into the tracheas of rabbits, found, after twenty-four hours, white, isolated areas in the bronchi and alveoli made up of round cells among which were the bacilli. Twenty-fours hours later these were necrotic and epithelial proliferation had begun. Thus we find, in marked contrast to one another, a liquefactive necrosis which we recognize under the more common term of suppuration, formation of fibrous tissue and a necrosis without liquefaction—three distinct processes differing from one another anatomically and histologically, and having for their etiological factor the poisonous substances present in the bodies of the dead tubercle bacilli.

That a multiplicity of lesions which differ in the amount of exudation and fibrin formation can result from the action of living tubercle bacilli, and that such lesions possess all transitional forms and stages from one to another, has been proven by the researches of Falk (10) under the guidance of Lubarsch. He found from many experiments upon animals and the examination of a large number of tissues from cases of human tuberculosis that the exudation and resulting formation of fibrin were dependent upon many factors, chief among which was the virulence of the bacillus. In support of this, fibrin was found to be absent both in the lesions produced by dead tubercle bacilli and those produced by bacilli of lessened virulence. That the tissue or animal resistance plays a part in the resulting phenomena of exudation was made manifest when it was learned that fibrin was generally absent in the tubercles produced in rabbits by pure cultures of living tubercle bacilli, and that it was constantly present in the tubercles produced in a similar manner in guinea-pigs, whose susceptibility to tuberculosis is universally known. The frequent presence of fibrin in genuine tubercle nodules in human beings

has been demonstrated by Werneck de Aquilar (44) in Baumgarten's laboratory.

The possibility of a further variation in the lesions produced by the bacillus of tuberculosis dependent upon the amount of degeneration or necrosis effected has been recognized by very few observers. R. Kockel (11) in the experimental tuberculosis of guinea-pigs found insular necroses in the liver which showed the following features: Twelve hours after the injection, areas were found which showed necrotic, shrunken, glistening liver cells with small nuclei. They were located generally adjacent to the radicles of the portal vein, and these were filled with polynuclear leucocytes among which the bacilli were present. Two days after inoculation the necrotic cells were still present adjacent to thrombi in the portal branches, in which proliferation of the endothelium of the vessel, as shown by the presence of karyokinetic figures, had resulted. These foci of necrosis the author believed to be due to plugging of the vessel, and in support of this view he gives the following reasons: 1. He cites the opinion of Schmorl (12) relative to the necroses observed in puerperal eclampsia, who believed such areas of cell death were produced in a similar manner. In this connection it must be noted that Lensden (13) takes exception to Schmorl's explanation of the genesis of the necroses observed in eclampsia, Lensden attributing the necroses to the action of some toxic substance in circulation in the blood. 2. Kockel mentions the experiments of Francken (14), Naunyn (15) and Schmorl (16), with the injection of substances capable of producing coagulation of the blood, in which experiments similar areas of necrosis were observed. 3. As a control Kockel employed an indifferent substance, grits (*Gries*), and produced in the livers of guinea-pigs areas of necrosis which differed in no respect from those found following the injection of tubercle bacilli, those occurring in eclampsia, or those caused by the injection of substances which promote fibrin formation. The liver cells in the areas produced by these different methods were much shrunken, stained strongly with eosin and possessed nuclei smaller than normal which stained less intensely or not at all.

It is important to remember, and we are reminded of the fact by

Leusden, that according to Ziegler (17) ischæmic necrosis does not occur in the liver unless radicles of both hepatic artery and portal vein are obstructed.

Previous to Kockel, necroses in the liver in connection with tuberculosis had been described by Pilliet (18), who found and described necroses in the livers of guinea-pigs, but believed that they had no specific relation to the bacillus of tuberculosis. In the human being Leredde (19) has described multiple visceral necroses in acute and subacute phthisis. Tubercle bacilli, however, were not found in the areas, the areas were located invariably around the central veins of the lobules, and from the description given and the accompanying illustrations it is difficult to assume that the lesions could be explained as the effect of chronic passive hyperæmia.

While we agree with Baumgarten that the usual effect of the tubercle bacillus upon the tissue is to produce a tubercle with a combination of exudative and proliferative changes, it seems evident from the lesions about to be described that the primary effect may be, at least in exceptional instances, one of necrosis combined with exudation and resulting fibrin formation; in other words, a coagulation necrosis. As instances of this, the following cases are presented.

Case I. Abstract of clinical history. G. S., a woman, colored, aged 60 years, was admitted to the Cook County Hospital, March 21, 1894. She complained of a cold and cough which had lasted for four weeks. Bronchophony and bronchial breathing, with marked dullness over both lower lobes posteriorly, were obtained upon examination of the chest.

Death took place April 19. The autopsy, made two days later, the body having been kept at about freezing temperature in the meantime, showed chronic vegetative endocarditis, chronic tuberculosis of both lungs with cavity formation and passive hyperæmia of the liver and kidneys. The spleen was small and hard, and showed no lesions of tuberculosis to the naked eye.

The lesions found microscopically in the spleen in this case serve as a type for those present in each of three cases, and are therefore described first.

Under a low power * attention immediately became directed to areas

* Obj. 16.0 mm., apr. 0.30, tube length 160 mm., ocular 4, diam. 62. Zeiss.

in which the nuclear staining was imperfect (Plate LIV, Fig. 1). These were so numerous that scarcely a field could be obtained which did not contain one such area, and many fields were found which contained two or three. In size the largest equaled in width one-third the breadth of the field under this power; an area of average size approximated closely the size of the normal renal glomerulus of an adult.* In serial sections, cut in paraffin and from 4 to 6 μ thick, the same area was found present on an average in twenty sections. As seen with this amplification in sections stained with hæmatoxylin and eosin, the eosin stain predominated and, upon this as a background, dust-like granules, darkly stained with hæmatoxylin, were distributed.

No round cell infiltration existed about any of these areas, nor could any fixed position of the areas be made out as regards either the blood-vessels (small arterioles) or the Malpighian corpuscles. Occasional areas were found situated close to one of the more minute of the radicles of the splenic artery, but at no time could the invasion of a Malpighian corpuscle or the presence of an area directly within such a corpuscle be found.

Under a higher power,† the dust-like granules seen before became more distinct as intensely staining, oval or round bodies of varying sizes, occupying mainly the central part of the area. The larger ones appeared highly refractile. All were smaller than cell nuclei. Their position, size and staining reactions left no doubt that they were the remains of nuclei, chromatin granules, resulting from nuclear fragmentation. Among these granules many distorted and shrunken nuclei, long drawn out, wedge-shaped, curved, or with dumb-bell-like enlarged ends, were found. These, although most numerous at the periphery, were also found here and there throughout the area. They exhibited all degrees of intensity of staining, but generally stained darkly. No cell bodies could be made out for these nuclei. Here and there in the areas were found cells with oval or round nuclei, which stained faintly or not at all with the nuclear dye.

A few cells with characteristic nuclei marking them as polymorphonuclear leucocytes were frequently observed in the peripheral zones, but in some areas these were entirely wanting. The transition between necrosis and the surrounding normal tissue was quite gradual. In the immediately surrounding tissue there were occasional cells possessing nuclei which stained poorly or not at all, surrounded by cells all of which

* Eckardt gives the diameter of a normal glomerulus in an eighteen-year-old girl, as 189.88 μ. Virchow's *Archiv*, cxiv (1888), 227.

† Obj. 2.0 mm., apr. 1.30, tube length 160 mm., eyepiece 8, diam. 1000. Zeiss.

stained in the usual manner. With the Weigert fibrin stain, fibrin was always found in these areas as a fine network, not only at the periphery but pervading the area throughout. In the central part the bands were often heavier; in the periphery the fine threads extended out among the unchanged cells. No evidences of thrombosis were found in adjacent capillaries or larger blood-vessels. To demonstrate the presence of the bacillus of tuberculosis, sections were cut in celloidin, which was then removed with clove oil. The sections were then fixed upon a slide by means of Mayers' albumin fixative or Schallibaum's collodion and clove oil mixture, stained with the ordinary carbol-fuchsin solution by heating for five or ten minutes, decolorized with 20 per cent sulphuric acid, followed by alcohol until a pinkish hue was obtained, and finally counterstained with methylene blue. By this method tubercle bacilli were invariably found—not in large numbers, but always a few in an area, and most numerous in the peripheral parts of the necrosis.

A careful examination by means of serial sections was made for multinuclear giant cells, but these were invariably wanting, nor were any areas found which presented for a part or the whole of their circumference cells with the vesicular nuclei commonly described in connection with the lesions of tuberculosis as "epithelioid cells."

A careful search for other bacteria was made by the following method. The sections fixed upon the slide were stained with methylene blue, washed slightly in water, cautiously allowed to dry, dehydrated with ether and clarified with xylol. This was found peculiarly applicable in that none of the blue was removed in dehydrating or clarifying. Results were negative. In the sections stained by the fibrin method also, no micrococci or other bacteria were found.

In the liver were found microscopically the following lesions: Situated within or occupying to a large extent the interlobular connective tissue, areas similar to those in the spleen were found. These differed from those in the spleen in that a slight zone of round cells, mainly polymorphonuclear leucocytes, were present around the periphery. The invasion of the area of necrosis by these leucocytes was generally slight and seldom extended throughout the area. The necrotic liver cells and fibrin made up the main part of the area (Plate LIV, Fig. 2). Giant cells were not found, but tubercle bacilli were easily demonstrated.

Case II. Abstract of clinical history. R. C., male, colored, aged 25 years; was admitted to the Cook County Hospital, February 21, 1894, with a history of illness which began with cough, fever and general weakness. At the date of entrance a painful cough, with considerable expectoration, loss of appetite and occasional vomiting were the main symp-

toms. A few rales were present over the whole chest; the abdomen was tender to pressure, and the spleen palpable. Death resulted March 7.

At the autopsy there were found a tubercular peritonitis with serofibrinous exudate; tuberculosis of the mesenteric, anterior mediastinal and bronchial glands; chronic tuberculosis with marked thickening of the right pleura; miliary tuberculosis of the upper lobe of the left lung, and a large and soft spleen adherent to the diaphragm at a point opposite to which the bronchial glands were adherent on its upper surface. The liver and kidneys on section showed nothing remarkable. Microscopical examination of the spleen showed areas similar to those in Case I, but they were not so numerous; in all other respects they were identical. In the liver, on the other hand, typical microscopic tubercles were present.

Case III. Abstract of clinical history. M. O'D., a woman, white, 48 years of age and single; was admitted to the Cook County Hospital, October 27, 1894, complaining of vomiting, dizziness, excessive perspiration, insomnia and occasional cough. On examination the relative cardiac dullness was found slightly increased, and a systolic murmur was heard over the lower part of the sternum. Liver dullness was increased, and some œdema of both lower extremities was present. Death occurred March 14.

The autopsy record has interest only in so far as it concerns the retroperitoneal lymph glands. There was present an unbroken chain of enlarged glands, extending from the aortic arch to opposite the sacrum. They were largest below; above they were pigmented. The largest was the size of a pigeon's egg. No marked adhesions existed between adjacent glands or the surrounding tissue. On section they all with one exception presented a smooth surface, of an even pale flesh tint throughout. The one exception was found in a small gland near the head of the pancreas, which contained calcified areas. No eroded bone was found in an examination of the vertebræ, sacrum and innominate bones.

Microscopic Examination. Situated beneath the capsule of the gland, and never extending to any great depth into the glandular substance, are many areas similar to those described in the spleen of Case I. They differ in that they are more irregular in outline and frequently confluent (Plate LIV, Fig. 3). These areas showed similar bizarre forms of cells and nuclei, similar nuclear fragments, absence of any peripheral round cell infiltration as well as absence of giant cells. Located here and there in the surrounding glandular substance, and without any fixed relation to the areas of necrosis, were found what appeared with a low power to be large cells, or possibly giant cells. Under a higher power these resolved

themselves into closely approximated nuclei surrounded by a homogeneous, highly refractile membrane. The nuclei were always few in number, never showed any central necrosis, and often no peripheral arrangement. For these reasons they were regarded as phenomena resulting from proliferation of the endothelium of lymph channels. This probability was further increased by finding, after considerable search, occasional leucocytes in the centre of some of the groups. Occasional large lymph cells were seen and some of these possessed a nucleus with a star-shaped mitotic arrangement of the chromatin elements. Tubercle bacilli were found in these areas, but they were not numerous. Other bacteria were not found.

That such areas of focal necrosis as we have seen to occur in the spleen in the most typical form are rare as a lesion of tuberculosis is to a slight extent substantiated by the researches of Manicatide (20). This writer examined the spleen in twelve cases of tuberculosis in children. In most of the cases the tuberculosis was visible to the naked eye; in two instances the tubercles were small, and in one of these no bacilli were found. In all of the other ten instances, typical tubercles possessing epithelioid cells and giant cells are spoken of.

That the areas in our three cases are areas of genuine focal necrosis is further substantiated by comparing these lesions with other observed forms of focal necrosis from various causes.

The substances ricin and abrin, products isolated respectively from the castor bean and the jequirity bean, studied by Warden and Waddell (21), Kobert and Stillmark (22), S. Martin (23), Hellin (24), Ehrlich (25), Kobert (26), S. Flexner (27), and Werhovsky (28), were found by Flexner to produce marked focal necroses in the lymphatic tissues with fragmentation of nuclei, also coagulation necrosis of liver cells and capillary endothelium in the liver and necrosis of the intestinal epithelium. Liver cells were found devoid of nuclei or the nuclei appeared in fragments. The cells sometimes were broken up into a reticulated mass, which reacted to Weigert's fibrin stain. More frequently the cells retained their shape but failed to stain with nuclear dyes. Ehrlich found marked necrosis produced by abrin at the point of inoculation with loss of hair from the surrounding skin, and came to the conclusion that abrin has a peculiar specific action upon the skin in this respect. Werhovsky in his experiments upon rabbits with abrin concluded that the greatest changes were produced in the cardiac muscle and the lining of the ali-

mentary canal. In the heart, swelling and fatty metamorphosis of the muscle fibres were observed; isolated foci of necrosis in the liver were not noted, and such changes as were found in the liver were attributed by him to the passive hyperæmia resulting from cardiac weakness.

Foci of necrosis in connection with diphtheria were first noticed by Bizzozero (29) in the spleen, mesenteric glands, and Peyer's patches. We are indebted to Oertel (30) for the first complete description of the histological changes which take place in these foci in human diphtheria, and to Welch and Flexner (31) and Babès (32) for concise accounts of similar changes produced in experimental diphtheria. By these authors focal necroses were described in the liver, spleen, lymph glands, heart muscle, kidney and intestines. Bulloch and Schmorl (33) have described areas of degeneration in the submaxillary gland in diphtheria in which fibrin was demonstrated; Dubief and Bruhl (34) produced necroses in the livers of guinea-pigs with experimental diphtheria; Courmont, Doyen and Paviot (35), necroses in the livers of dogs, produced by the diphtheria toxins. The necroses described by these various authors consist of minute areas of nuclear fragmentation, with entire or partial dissolution and disappearance or great distortion of the fixed cells of the part. The nuclear remains stain darkly and give a dust-like appearance to the area. There were also found areas in which the cells retained their shape but stained imperfectly. In addition to these changes Barbacci (36) in a recent article has described œdema, hæmorrhage and hyaline degeneration of the follicles of the spleen, and only the occasional presence of fibrin; in the lymph glands a predisposition for the necroses to occur in the peripheral parts of the gland and in the germinal areas, also hyaline degeneration of the vessels, cell elements and stroma; in the liver, changes were described in the nuclei of the liver cells, but no actual foci of necrosis.

Foci of necrosis have been found in the liver in cases of puerperal eclampsia by Pilliet (37), Gerdes (38), Lubarsch (39), Schmorl (16), and Leusden (13). The liver cells were necrotic and stained more intensely with eosin than the surrounding liver cells; in places they showed irregular, indefinite outlines with shrunken nuclei.

Flexner (27) found necroses in the viscera of rabbits into which intravenous injection of the blood serum of dogs had been made. They were present in the liver, spleen and kidney; in the spleen marked nuclear fragmentation resulted, in the liver areas of coagulation necrosis.

The so-called lymphoid nodules occurring in the liver in typhoid fever, described by Friedreich, E. Wagner and others, have been shown by Reed (40) to be areas of necrosis into which the polymorphonuclear

leucocytes have wandered, and were produced experimentally by the last named author by injecting pure cultures of the typhoid bacillus into the mesenteric veins of rabbits. The areas of necrosis, according to Reed, are frequently oval or irregular in outline, occupying a space equivalent to a few liver cells or the larger part of a lobule, are situated in any part of the lobule, and show various signs of cell death or degeneration. In places the shape of the cell and cell rows is well preserved, but nuclear fragmentation and deficient stain absorption are present. In other areas the liver cells have lost their shape, show pale and swollen nuclei and may contain polynuclear leucocytes. In still other areas the liver cells have entirely disappeared.

Areas of necrosis have been noted in the liver as the result of hog cholera infection by Welch in 1889 and by other observers (41), but a detailed account of the minute changes taking place is apparently wanting.

Foci of necrosis have also been noted in the liver in cases of acute lobar pneumonia and in experimental pneumococcus infection by Welch (42). In these, the nuclei of the liver cells were in large part absent or fragmented, the cell body opaque or more refractive than normal or containing fat globules. Polynuclear leucocytes had invaded the areas from the edges.

Wright (43) has recently given us an account of the earlier histological changes which take place in the nodules of experimental glanders in guinea-pigs, in which the changes noted are very similar to those already described in other forms of focal necrosis. The foci were observed in the liver, spleen, lymph glands, testicle, peritoneum and adrenals as minute greyish points, or larger, somewhat yellowish areas, and in all localities showed microscopically essentially the same changes. Marked necrosis or degeneration of the fixed cell elements, as shown by the various degrees of cell dissolution, deficient staining, and a considerable quantity of nuclear remains in the shape of darkly staining chromatin granules or balls, was found, with a slight infiltration of polynuclear leucocytes which likewise had undergone necrosis or degeneration. From these findings he takes exception with Tedeschi to the heretofore accepted views of Baumgarten concerning the histogenesis of the nodules of glanders, in that nothing was found which would indicate that a primary cell proliferation had occurred, and he comes to the conclusion that the first effect of the bacillus of glanders upon the tissues is to produce, not cell proliferation, but cell death.

CONCLUSIONS.

1. It must be accepted from a comparison between the histological changes described in the focal necroses due to abrin and ricin, diphtheria, eclampsia, blood-serum intoxication, typhoid fever, lobar pneumonia and glanders, and those described here in three cases of tuberculosis, that the last are also instances of focal necrosis.

2. It may be claimed that the areas of necrosis in the three cases of tuberculosis had their origin in ischæmia, for the reason that they were numerous in the spleen and were not found in the kidney. Against such an explanation of their causation are the facts: that other forms of necrosis affect frequently the spleen and seldom the kidney; that thrombosis of vessels or any fixed relation of the areas in the spleen to blood-vessels could not be established; that the tubercle bacilli were invariably present; and lastly and of no slight importance, that these necroses were all present in cases of marked marasmus.

3. That the areas of necrosis in these three cases might be phenomena resulting from a mixed infection with pyogenic bacteria is possible, but no groups, colonies or even single bodies could be found which answered to the requirements in shape or staining properties for such bacteria. Even were it the case that the focal necroses here presented are the histological lesions of a septicæmia occurring as a complication of tuberculosis, the presence of the bacillus of tuberculosis in the areas of necrosis would still require explanation.

4. Lastly, it is not unreasonable to suppose that a diminished resistance on the part of the tissues incidental to marasmus is an important factor in the production of these necrotic lesions.

DESCRIPTION OF PLATE LIV.

Fig. 1. Area of necrosis in the spleen. Case I. × 62.

Fig. 2. Area of necrosis in the liver. Case I. Weigert's fibrin stain. 1, heavy bands of fibrin. 2, finer bands of fibrin. 3, degenerate liver cell nucleus. 4, leucocyte. × 1000, reduced one-third.

Fig. 3. Necroses in lymph gland. Case III. × 62.

BIBLIOGRAPHY.

1. Baumgarten.—(Quoted by Falk. See reference 10.)

2. Orth.—Lehrb. d. spec. path. Anat., i, 954, Berlin, 1887.

3. Koch.—Fortsetzung der Mittheilungen über ein Heilmittel gegen Tuberculose. *Deutsche med. Wchnschr.*, xviii (1891), 101.

4. Wyssokowicz.—Separat-Abdruck aus "Mittheilungen aus Dr. Brehmer's Heilanstalt für Lungenkranke in Görbersdorf." Wiesbaden, 1890.

5. Prudden and Hodenpyl.—Studies on the action of dead bacteria in the living body. *New York Med. Jour.*, liii (1891), 637, 697.

6. Vissman.—Wirkung todter Tuberkelbacillen und des Tuberculins auf den thierischen Organismus. Virchow's *Archiv*, cxxix (1892), 163.

7. Straus and Gamaleia.—Contribution à l'étude du poison tuberculeux. *Arch. de méd. expér. et d'anat. path.*, iii (1891), 705.

8. Masur and Kockel.—Zur Kenntniss von der Wirkung todter Tuberkelbacillen. Ziegler's *Beiträge*, xvi, 256.

9. Abel.—*Deutsche med. Wchnschr.*, 1892, 482.

10. Falk.—Ueber die exsudativen Vorgänge bei der Tuberkelbildung. Virchow's *Archiv*, cxxxix (1895), 319.

11. Kockel.—Beitrag zur Histogenese des miliaren Tuberkels. Virchow's *Archiv*, cxliii (1896), 574.

12. Schmorl.—*Centralbl. f. allg. Path. u. path. Anat.*, ii, 803.

13. Leusden.—Beitrag zur pathologischen Anatomie der Puerperaleklampsie. Virchow's *Archiv*, cxlii (1895), 1.

14. Francken.—Ein Beitrag zur Lehre von der Blutgerinnung im lebenden Organismus und ihren Folgen. Inaug. Dissert., Dorpat, 1870.

15. Naunyn.—Untersuchungen über Blutgerinnung im lebenden Thiere und ihre Folgen. *Arch. f. exp. Path. u. Pharmakol.*, i (1873), 1.

16. Schmorl.—Pathologisch-anatomische Untersuchungen über Puerperaleklampsie. Leipzig, 1893.

17. Ziegler.—Lehrb. d. spec. path. Anatomie, ii, 574, Jena, 1892.

18. Pilliet.—Etude sur la tuberculose expérimentale et spontanée du foie. Thèse de Paris, 1892; and Étude sur la tuberculose du cobaye, in Verneuil's Etudes expér. et clin. sur la tuberculose.

19. Leredde.—Nécroses viscérales multiples dans la tuberculose humaine aigue et subaigue. *Arch. de méd. expér. et d'anat. path.*, vii (1895), 87.

20. Manicatide.—Étude sur la rate chez les enfants tuberculeux. *Rev. mens. d. mal. de l'enf.*, xiv (1896), 88.

21. Warden and Waddell.—The Non-Bacillar Nature of Abrus Poison. Calcutta, 1884.

22. Kobert and Stillmark.—Arb. d. pharmakol. Inst. zu Dorpat, iii, 59, Stuttgart, 1889.

23. Martin, S.—Report on Proteid Poisons, with special reference to that of the Jequirity (Abrus precatorius). *Brit. Med. Jour.*, July 27, 1889, 184.

24. Hellin.—Der giftige Eiweisskörper Abrin und seine Wirkung auf das Blut. Inaug. Dissert., Dorpat, 1891.

25. Ehrlich.—Exp. Untersuch. üb. Immunität. I. Ueber Ricin. *Deutsche med. Wchnschr.*, xvii (1891), 976.

26. Kobert.—Lehrb. der Intoxikationen. Stuttgart, 1893.

27. Flexner.—The pathological changes caused by certain so-called Toxalbumins. *Med. News*, Aug. 4, 1894, 116; and The Histological Changes produced by Ricin and Abrin Intoxications. *The Journal of Experimental Medicine*, ii (1897), 197.

28. Werhovsky.—Beiträge zur path. Anat. d. Abrinvergiftung. Ziegler's *Beiträge*, xviii, 115.

29. Bizzozero.—Beiträge zur path. Anat. d. Diphtheritis. *Oesterr. Med. Jahrb.*, 1876, ii, 207.

30. Oertel.—Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung. Leipzig, 1887.

31. Welch and Flexner.—The Histological Changes in Experimental Diphtheria. *Johns Hopkins Hosp. Bull.*, ii (1891), 109; and The Histological Lesions produced by the Tox-Albumin of Diphtheria, Ibid., March, 1892.

32. Babès.—Untersuch. üb. d. Diphtheriebacillus u. d. experimentelle Diphtherie. Virchow's *Archiv*, cxix, 460; also Gewebsveränderungen bei experimenteller Diphtherie. *Verhandl. d. internat. med. Congresses.* Berlin, 1890.

33. Bulloch and Schmorl.—Ueber Lymphdrüsenerkrankungen bei epidemischer Diphtherie. Ziegler's *Beiträge*, xvi, 247.

34. Dubief and Bruhl.—Note sur une altération des cellules hépatiques dans la diphtérie expérimentale. *Compt. rend. Soc. de biol.*, Par., 9. s., iii (1891), 135.

35. Courmont, Doyen and Paviot.—Des lésions hépatiques expérimentales engendrées par la toxine diphtérique. *Arch. de physiol. norm. et path.*, 5. s., vii (1895), 687.

36. Barbacci.—Ueber die feineren histologischen Alterationen der Milz, der Lymphdrüsen und der Leber bei der Diphtherie-Infection. *Centralbl. f. allg. Path. u. path. Anat.*, vii (1896), 321.

37. Pilliet.—Le foie des éclamptiques. *Gaz. hebd. de méd.*, 1890, 2. s., xxvii, 349.

38. Gerdes.—Ueber den Eklampsiebacillus und seine Beziehungen zur Pathogenese der puerperalen Eklampsie. *Deutsche med. Wchnschr.*, xviii (1892), 603.

39. Lubarsch.—Zur Lehre von der Parenchymzellenembolie. *Fortschr. d. Med.*, xi (1893), 805, 845; also *Centralbl. f. allg. Path. u. path. Anat.*, ii, 803.

40. Reed.—An Investigation into the so-called Lymphoid Nodules in Abdominal Typhus. *The Johns Hopkins Hospital Reports*, v (1895), 379.

41. Welch.—Preliminary Report of Investigations concerning the Causation of Hog Cholera. *The Johns Hopkins Hospital Bulletin*, Dec., 1889.

Welch and Clement. Remarks on Hog Cholera and Swine Plague. Proc. of 30th Annual Convention of U. S. Veter. Med. Assoc., and 1st Veter. Congr. of America (1893), p. 206.

Moore.—A non-motile pathogenic bacillus closely resembling the bacillus of Hog Cholera, found in the lung and spleen of a pig. Bulletin No. 3, 1893, p. 35, Bureau of Animal Industry.

42. Welch.—The Micrococcus Lanceolatus with special reference to the Etiology of Acute Lobar Pneumonia. *Johns Hopkins Hosp. Bull.*, iii (1892), 134.

43. Wright, J. H.—The Histological Lesions of Acute Glanders in Man and of Experimental Glanders in the Guinea Pig. *The Journal of Experimental Medicine*, i (1896), 1.

44. Werneck de Aquilar.—Ueber Fibrinbildung bei den verschiedenen anatomischen Producten der Tuberkulose. Arbeit. aus d. path.-anat. Inst. zu Tübingen, ii (1896), 245.

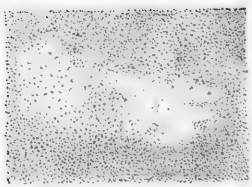


FIG. 1.

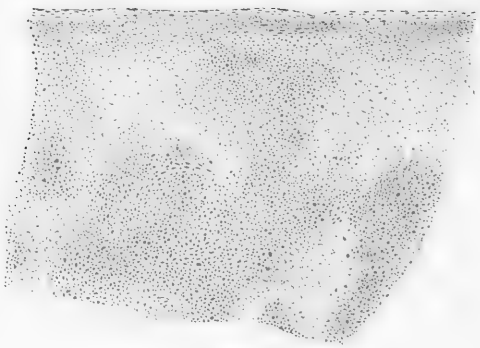


FIG. 3.

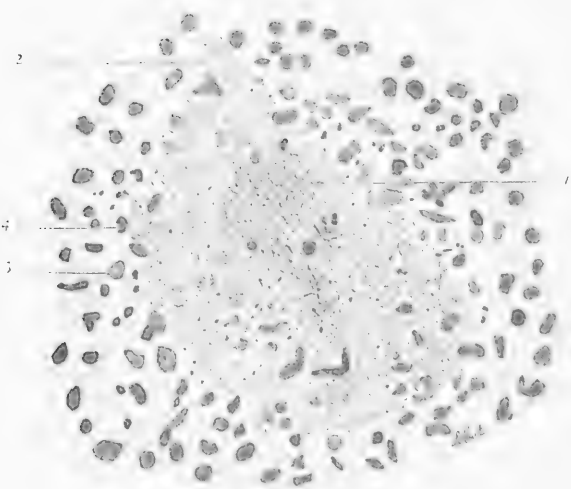


FIG. 2.

TECHNICAL NOTE.

I. A MODIFIED FIXING FLUID FOR GENERAL HISTOLOGICAL AND NEURO-HISTOLOGICAL PURPOSES.
II. A STAINING COMBINATION OF GENTIAN VIOLET AND PICRO-ACID FUCHSIN.

By A. P. OHLMACHER, M. D.

(From the Pathological Laboratory of the Ohio Hospital for Epileptics, Gallipolis, O.)

I.

In routine work in neural pathology it is often desirable to harden portions of the central nervous system in bulk, and to preserve the tissue in such a way as to make it suitable for subsequent microscopical study. The necessity of precise fixation of delicate histological details is especially felt in the new line of cytological pathology in which the effects of certain diseases of the nervous system are to be sought in alterations of the ganglion cells. To meet these indications a comparatively cheap fixing fluid is demanded which will penetrate considerable masses of tissue with rapidity, which will produce a desirable degree of hardening, which will permit of subsequent staining by a variety of dyes, and, above all, which will faithfully preserve the most delicate structural details. The necessity for a reagent of this kind has been felt by the workers in the Pathological Institute of the New York State Hospitals* in the treatment of the large amount of neurological material which is finding its way to this laboratory, and at present Lang's solution of corrosive sublimate is used, though it has not proven entirely satisfactory. A fresh human brain is subdivided after Meynert's method, placed in the sublimate solution for 48 hours, washed in running water, and treated with ascending alcohols till a strength of 80 per cent is reached. Tincture of iodin is added to exhaust the sublimate. The chief drawback to this otherwise excellent

* Dr. Ira Van Gieson, director of the laboratory, kindly furnishes me the details of his method of treating the brain, and permits me to refer to them.

procedure lies in the tardiness with which the sublimate penetrates, though Dr. Van Gieson informs me that a good fixation of the cerebral cortical layer is attained.

For the past six years it has been the practice of the writer to employ Carnoy's chloroform-acetic alcohol mixture* as a fixing fluid for general purposes of normal and pathological histology. This medium is comparatively cheap, cleanly and convenient; it affords a reliable fixation, and penetrates more rapidly than any fluid known to me. The rapidity of penetration is doubtless due to the chloroform, which was added for this purpose by Carnoy as an improvement on his original acetic-alcohol mixture. The fixation of nervous tissue by the reagent has not seemed sufficiently precise, and after hearing of Dr. Van Gieson's method of handling neurological material, the idea of combining corrosive sublimate and Carnoy's solution was suggested.

A careful trial of this combination has shown it to be a very valuable fixing and hardening medium. It has been used upon a variety of fresh normal tissues from the human body and from animals, upon the brain and spinal cord of man and of animals (sheep, cat and rabbit), and upon several kinds of pathological tissue.

Comparative tests with Lang's solution of corrosive sublimate upon the cerebral hemispheres of the sheep, cat and rabbit show the sublimate-Carnoy fluid to penetrate at least twice as rapidly and to afford a more rapid hardening. There is a moderate shrinkage of the brain mass in the fluid, slightly in excess of that produced in the aqueous solution, but upon subsequent treatment with alcohol the shrinkage is more pronounced in the material fixed in Lang's mixture. Compared with formaldehyd-Müller's fluid (4 parts of 40 per cent formaldehyd to 100 parts of Müller's fluid), a more pronounced shrinkage in the bulk occurs, and this is again largely equalized by the alcoholic treatment necessarily following both fixing fluids.

In the matter of precise fixation, the combination of sublimate with

*The formula for Carnoy's (second) fixative (Lee's Microtomist's Vade Mecum, 4th Edition, p. 45) is:

Glacial acetic acid	1 part
Absolute alcohol	6 parts
Chloroform	3 parts

the chloroform-acetic alcohol mixture appears to fulfil every indication. This is the case with pieces of various organs and tissues for general histological study, for cytological study, and for neuro-cytological investigation. Tested beside Flemming's fluid on such delicate objects as the regenerating tadpole's tail, the preservation of the cytological details is equally good with the modified mixture. Pieces of the cerebrum, cerebellum and cord, cut from these portions after fixation *in toto*, and stained by various dyes including gentian violet, safranin, and fuchsin and methylene blue (after the Nissl process), furnish most excellent pictures of cellular structure. The preservation of such details as the nuclear framework and chromophilous plates in the ganglion cells, and the chromatin network in the cerebellar granules, is remarkably good. A comparison of the fixation of the fresh human cerebrum and cerebellum in bulk, and cut into pieces, shows no appreciable difference.

After experimenting with various modifications of Carnoy's original formula, a fluid made as follows has been adopted:

Anhydrous alcohol	80 parts
Chloroform	15 parts
Glacial acetic acid	5 parts
Corrosive sublimate	to saturation.

The alcohol employed in the mixture is made by dehydrating 95 per cent alcohol by means of anhydrous copper sulphate. About 20 grammes of powdered corrosive sublimate are required to slightly over-saturate 100 ccm. of the fluid.

Ordinary pieces of tissue are sufficiently fixed in fifteen minutes to half an hour. For fixation of the human encephalon, Meynert's section is adopted, including the removal of the cerebellum at the peduncles with a hemi-section of the hemispheres, and a hemi-section of the brain mantle. The pia is generally removed from one cerebral hemisphere and allowed to remain on the other before the pieces are placed in the fixing fluid, as is the practice in Dr. Van Gieson's laboratory. These portions can be fixed in their entirety, or the axis, together with one cerebral and one cerebellar hemisphere, can be fixed in the sublimate mixture, while the remaining pieces can be treated

by some other process, as, for instance, formaldehyd-Müller's fluid. For the fixation of the subdivided brain an immersion of eighteen to twenty-four hours in the sublimate-Carnoy fluid has been allowed with good results, though a longer time may be found desirable in some cases.

After fixation the tissue is washed by a bath of 80 per cent alcohol and placed in alcohol of this strength for preservation. The removal of the sublimate is hastened either by adding gum camphor to the alcohol, or by adding tincture of iodin from time to time, until its yellow color is no longer discharged. For microscopic purposes the fixed tissue is removed to absolute alcohol, or 95 per cent alcohol, for dehydration, cleared in cedar-wood oil, or bergamot oil (for very delicate material), and embedded in paraffin. Where an early microscopic examination is desirable it is not necessary to wait for the extraction of the sublimate, for this can be effected by treatment with tincture of iodin after sections are affixed to the slide, as advised by Heidenhain.*

Only enough of the fluid to cover the specimens is required; for example, the human brain axis, with one-half of the mantle and one cerebellar hemisphere, lying on absorbent cotton in a suitable dish, can be fixed by 1000 to 1500 ccm. of the reagent. It is quite possible that the fluid can be used more than once, but it has not seemed desirable to risk this when the value of a well-fixed brain was weighed beside the cost of the fluid. As soon as the tissue is placed in the fixing fluid it begins to whiten and the hardening rapidly develops. Portions of the brain preserved by this method make very pretty gross objects in the purity of their whiteness.

The solution is very corrosive and extremely hard on the living skin, so that care must be exercised in handling tissue during fixation. The usual precautions about metallic contact which apply to all sublimate solutions must of course be observed.

*For a review of Heidenhain's method see Ohlmacher, *Medical News*, Nov. 19, 1892, pp. 573-576.

II.

One of the most widely used staining combinations for purposes of pathological histology is Van Gieson's hæmatoxylin picro-acid fuchsin method, which, aside from its general utility, affords a valuable special stain for axis cylinders. Hæmatoxylin, especially in conjunction with picro-acid fuchsin, does not uniformly afford that delicacy of nuclear staining which can be obtained by certain of the anilins. It does not, for instance, compare in precision with gentian violet used by the "indirect" method. The desirability of a combination of gentian violet and picro-acid fuchsin occurring to me, it was found that the two stains could be readily combined, and that an eligible staining process was the result. This process gives good results in tissue fixed in formaldehyd solutions, Carnoy's fluid, absolute alcohol, Flemming's fluid, and Hermann's mixture; though the staining after sublimate fixation is especially brilliant, as would be expected from the well known partiality of gentian violet for tissue fixed with this reagent. Fixation by the sublimate-chloroform-acetic alcohol mixture above described, followed by this stain, gives most beautiful results in successful preparations. Sections of the cerebellum fixed and stained in this way show a wealth of detail in plasmatic and nuclear structures. The special affinity of picro-acid fuchsin for axis cylinders is retained, and even accentuated; and the details of triple staining characterizing hæmatoxylin picro-acid fuchsin are all preserved. The method is to be practised as follows:

Stain (sections affixed to the slide) with Ehrlich's anilin-water gentian violet, 1 minute.

Drain excess of stain, and wash with water.

Treat directly with picro-acid fuchsin solution.

Wash thoroughly with water.

Wash with absolute alcohol.

Clear with clove oil and mount in xylol balsam.

The gentian violet solution must, of course, be in good condition. The picro-acid fuchsin is made by adding ½ per cent of acid fuchsin (Fuchsin S., Grübler) to a saturated aqueous solution of picric acid which has been diluted with an equal quantity of water. The dura-

tion of the counter stain varies slightly, but is usually only momentary. The washing with water should be sufficient to remove the traces of acid, which otherwise causes the specimen to fade. The treatment with absolute alcohol ceases as soon as clouds of color subside (generally not more than half a minute). Differentiation may be completed in the clove oil, under the microscope, providing it has not been sufficient with the alcohol.

ON A METHOD OF ISOLATING AND IDENTIFYING BACILLUS TYPHOSUS, BASED ON A STUDY OF BACILLUS TYPHOSUS AND MEMBERS OF THE COLON GROUP IN SEMI-SOLID CULTURE MEDIA.

By PHILIP HANSON HISS, Jr., M. D., Assistant in Bacteriology, College of Physicians and Surgeons, Columbia University; Assistant Bacteriologist to the New York City Health Department.

(From the Bacterial Laboratory of the College of Physicians and Surgeons, and the Research Bacteriological Laboratory of the Health Department of New York City.)

GENERAL CONSIDERATIONS.

The experience of all workers in bacteriology includes instances of the occurrence of unusual variations in the colony and growth characters presented by the same organism when transplanted from one medium to another of apparently the same composition and developed under the same conditions of temperature and moisture. When one considers the crude methods often employed to determine the reaction of a medium, and the changes so readily produced in media, especially those containing gelatin, by the exposure to heat in their preparation, these variations are not remarkable, and probably indicate no especial tendency on the part of the organism to vary, but only its natural appearance under the conditions present. Bearing in mind the value placed upon growth appearances, especially upon the form and size of colonies, in the differentiation of species, one is impressed, in reviewing the literature of bacteriology, with the laxity and paucity in descriptions of the mode of preparation and of the exact composition and reaction of the media in which these appearances occur. The differences in the media used by different observers may account to some extent for the discrepancy in accounts of the same organism and may also be responsible for its description as a new variety or species.

The observations of Baginsky, Rosenthal and Klie are of interest in this connection, especially as they call attention to the variation of colony forms in media of reduced consistence. These experimenters fail to record in detail the reaction of the media used.

Baginsky * in 1889, while examining stools from cases of cholera infantum, noted peculiar colonies showing ramifying outgrowth and forms not usual to Bacillus coli communis. From these appearances he thought that he had to deal with an undescribed organism, but careful study upon new plates showed it to be identical with Bacillus neapolitanus.

Werner Rosenthal † met with peculiar colonies which he found to be those of Bacillus coli communis. He experimented afterwards with the variation in the colony forms of Bacillus coli communis and Bacillus typhosus in media containing 2.5 per cent and 3.3 per cent of gelatin. He notes that his observations and those of Baginsky were made during the hot months.

Klie ‡ continued the experiments of Rosenthal and came to the conclusion that the temperature is of as much if not more importance than the percentage of gelatin, for it has three effects: increase of the rate of multiplication of the bacteria, increase of their motility, and its effect upon the consistence of the medium.

Kruse § considers that the finer structure of colonies does not show great constancy, and that growth characters depend especially upon the composition of the medium, its consistence, degree of alkalinity, and upon the growth conditions, especially temperature, and he also notes that through a reduction in the consistence of the gelatin, such as is caused by prolonged boiling, colony forms are influenced and "proteus-like" appearances may occur.

From such facts as these it seems fair to conclude that the form of colonies and the distribution of an organism on and in the various culture media are to a great extent purely mechanical problems, that they are the result of the resistance offered by the density of the medium, the motility or non-motility of the organism, and its rate and mode of increase under the given nutrient and temperature conditions.

The influence of the products of metabolism, and of the changes produced in the various constituents of the medium by the organism during growth, must not be lost sight of in this connection, for the presence of these products is often unfavorable for the further development of the organism, and this may show itself more quickly in a solid medium in which these products cannot diffuse rapidly, and become locally more concentrated, than in a fluid or semi-solid medium.

Too much stress therefore cannot be laid upon the necessity of a de-

* Baginsky, *Berlin. klin. Wochenschrift*, 1889, 996.

† Werner Rosenthal, *Deutsch. Arch. f. klin. Med.*, lv (1895), 513.

‡ Klie, Inaug. Diss., Erlangen, 1896, and *Centralbl. f. Baktr.*, xx (1896), 49.

§ Kruse, in Flügge's Die Mikroorganismen, 3te Aufl., p. 480, Leipzig, 1896.

tailed description of the constituents of the medium, the mode of preparation, the reaction and the temperature conditions under which the appearances noted occur, as a variation in any or all of these factors may cause differences in the appearance of the growth or the colony forms.

Having been thoroughly impressed with the importance of the above considerations, and believing that a profitable and scientific field of research lay in the comparative study of bacteria, especially motile forms, in media of carefully varied consistence and composition, obtained by varying the proportion of the constituents of our ordinary nutrient media, *i. e.* the gelatin, agar, peptone, glucose, alkali, acid, etc., the writer some fifteen months ago undertook experiments the results of which led him to the researches described in the present paper.

These researches have been limited to the study of Bacillus typhosus and members of the "colon group," and have been carried on in an attempt to establish a broad and practical differentiation between these two forms of organisms, based on their growth peculiarities in semi-solid media, especially in media becoming semi-solid between 30° and 40° C.

The term "colon group" is used broadly and includes members of both the colon and aerogenes groups, described by Kruse in the third edition of Flügge's "Mikroorganismen." According to Kruse, the most fundamental difference between these groups is the motility of the colon group and the absence of motility in the aerogenes group. Since some of the aerogenes group at times display motility, even this difference between these groups is not constant.

During these experiments it was found that cultures of Bacillus coli communis obtained from the different laboratories and other sources were widely different in their behavior in semi-solid media. It has therefore been necessary to divide them roughly into two groups, dependent to a great degree upon their difference in motility.

The absolute identification of the different members of the "colon group" is not a matter of paramount importance to us, since the question under consideration is not so much to differentiate one organism, for instance the true Bacillus coli communis, from Bacillus

typhosus, as to obtain a differentiation broad enough to include all members of the group which may be found associated with Bacillus typhosus in material subjected to investigation.

The cultures of Bacillus typhosus and of Bacillus coli communis and of members of the "colon group" at my command were from many different sources, some having been cultivated for years on artificial media, others being tested soon after isolation. Thirteen different typhoid cultures have been used uniformly throughout the experiments. Among the typhoid cultures is a culture from Prof. R. Pfeiffer of Berlin, which has been regularly used at the laboratories of the New York Department of Health in the serum diagnosis of typhoid fever, also a culture from Dr. Wyatt Johnston of Montreal used in the same work. Several of the cultures are from the Pathological Laboratory of the Johns Hopkins University and one recently from Kral's laboratory in Prag. Of the other cultures experimented with, one was obtained by spleen puncture, and several were isolated from the organs of patients dead of typhoid fever.

DIFFERENTIATION OF BACILLUS TYPHOSUS FROM THE BACILLI OF THE COLON GROUP BY TUBE CULTURE.

NON-MOTILE OR LESS ACTIVELY MOTILE DIVISION OF THE COLON GROUP.

The first cultures of the colon group under observation belonged distinctly to the less actively motile division, and their behavior and appearances in the various media experimented with will be described in detail and compared with the characters presented by the typhoid cultures under similar conditions, and then the active type will be considered.

The preliminary experiments were made with tubes of 10 per cent nutrient gelatin inoculated with B. typhosus and B. coli communis and placed in the incubator at 37° C. The tubes containing the typhoid growth, examined after 24 hours, showed a uniform clouding of the medium, while the medium containing B. coli remained clear, with flocculi scattered throughout and a slight deposit. As it seemed probable that these differences were due to the difference in motility of these organisms, a less fluid medium was tried to determine whether changes in consistence would increase these differences in appearance.

For this purpose an agar-gelatin mixture of 0.5 per cent of agar and 8 per cent of gelatin was adopted as the stiffening basis. The first medium of this kind tried contained 0.5 per cent meat extract, 0.5 per cent NaCl, 2 per cent peptone and 1 per cent glucose, and showed a reaction 1.5 per cent acid to phenolphthalein. The inoculated tubes were allowed to remain in the incubator at 37° C. for 24 hours. The cultures of B. typhosus showed a uniform clouding of the medium extending completely across the tube and downwards with even base, to a varying extent depending upon the time and depth of puncture, but generally becoming completely clouded within 24 hours. The growth along the lines of puncture was hardly more dense than throughout the rest of the medium. The colon cultures, on the other hand, developed a growth only along the line of inoculation, at times with some outspreading of isolated colonies. Later, in some parts of the medium, a local clouding often developed. Gas was formed, and in the wake of the ascending bubbles streaks and patches of growth occurred, giving in 24 to 48 hours a medium generally clear, with a varying number of these patches scattered throughout it.

The next experiments were made to determine the effect of different quantities of acid and alkali on the growth and action of the organisms, and also with the hope that the local clouding, which displayed itself in some of the colon cultures, would be suppressed by a certain percentage of one of these ingredients.

Fourteen media were made, containing 0.5 per cent meat extract, 0.5 per cent NaCl, 1 per cent peptone and 1 per cent glucose, and varying in reaction from neutral (phenolphthalein) to a reaction indicating 2.5 per cent of normal NaOH solution; and from neutral to 4 per cent of normal HCl solution, by 0.5 per cent increments. (See Table I, p. 683.)

Cultures of B. typhosus and of B. coli communis in these media showed that the maximum growth for both organisms occurred in media of the same reaction, *i. e.* containing between 1.5 per cent to 2 per cent of normal NaOH solution. In the case of the typhoid bacillus the media giving the optimum growth often showed a complete and uniform clouding within 18 hours, and always within 24 hours. The colon bacillus gave an abundant growth within 24 hours, and a marked outspreading of isolated colonies extending from the line of inoculation. Gas was always produced, and in the track of the bubbles streaks and masses of growth appeared.

As the percentage of alkali decreased or increased from the most favorable reaction, so the growth and rapidity of spreading decreased in the typhoid and the colon series. When the reaction became 1 per cent

acid, only a faint clouding occurred in the upper part of the typhoid tubes, and the growth along the puncture in the colon tubes became very meagre indeed. In both series growth did not take place in the media containing the higher percentages of acid, being generally inhibited in the media containing about 3.5 per cent normal HCl solution.

These media were also inoculated on the surface by means of a loop from broth cultures, and as such surface inoculations gave the most information in regard to the maximum growth and rapidity of spreading, especially with the typhoid cultures, tables recording observations of such development are given (Tables I and II, pp. 683, 684).

It was found by these experiments that any of the media which were uniformly clouded by the typhoid growth served very satisfactorily to differentiate B. coli from the typhoid bacillus, yet a tendency was shown by B. coli, in parts of the tube, to stimulate the typhoid growth.

Further experiments were, therefore, undertaken to limit if possible this tendency on the part of B. coli to cloud the medium, and as all the media heretofore used seemed too complex for definite conclusions in regard to the effect of a given ingredient on the growth of the organisms, the following scheme (Table III, p. 685) was adopted so as to be able to attribute as directly as possible an effect to its proper cause.

This scheme was as follows: The same gelatin agar mixture of 5 gm. of agar and 80 gm. of gelatin to the litre was taken, and to this was added 5 gm. of Liebig's beef extract and 5 gm. of common salt. This was tested, after boiling, for its reaction by means of phenolphthalein, and then brought to the "standard" reaction by the addition of normal NaOH or HCl solution. The reaction adopted as "standard" allows of 1.5 per cent of normal acid, or 15 cc. of normal acid to the litre, over the reaction indicating neutrality to phenolphthalein. This simplified medium brought to the standard reaction was taken as the basis of the series of media.

The series consisted of twenty-nine media. Seventeen of them forming an alkaline and an acid series, graded by differences of 0.5 per cent from standard to 4 per cent of normal NaOH solution, and from standard to 4 per cent of normal HCl solution. Four others of the media were a peptone series, and eight a glucose series (see Table III).

TABLE I.

B. TYPHOSUS. "H." SURFACE-DROP INOCULATION. NOV. 14, 1896. 4.30 P. M.

No.	Water.	Meat extract.	NaCl.	Peptone.	Glucose.	Agar.	Gelatin.	NaOH.	HCl.	18 hours. 37° C.	24 hours. 37° C.	48 hours. 37° C.
	cc.	gm.	gm.	gm.	gm.	gm.	gm.	cc.	cc.			
1	1000	5	5	10	10	5	80	25		Upper $\frac{2}{3}$ clouded.	Clouded throughout.	
2	"	"	"	"	"	"	"	20		Upper $\frac{5}{6}$ clouded.	Clouded throughout.	
3	"	"	"	"	"	"	"	15		Upper $\frac{4}{5}$ clouded.	Clouded throughout.	
4	"	"	"	"	"	"	"	10		Upper $\frac{2}{3}$ clouded.	Clouded throughout.	
5	"	"	"	"	"	"	"	5		Upper $\frac{1}{2}$ clouded.	Clouded in upper $\frac{7}{8}$.	Clouded throughout.
6	"	"	"	"	"	"	"	neutral.		Upper $\frac{1}{3}$ clouded.	Upper $\frac{1}{2}$ clouded.	Clouded throughout.
7	"	"	"	"	"	"	"		5	Upper $\frac{1}{4}$ clouded.	Upper $\frac{1}{3}$ clouded.	Upper $\frac{5}{6}$ clouded.
8	"	"	"	"	"	"	"		10	Slight clouding in upper portion.	Slight clouding in upper $\frac{1}{4}$.	Upper $\frac{1}{2}$ clouded.
9	"	"	"	"	"	"	"		15	Similar to No. 8, but less.	Same as 18 hrs.	Misty in upper $\frac{1}{3}$.
10	"	"	"	"	"	"	"		20	Similar to No. 9, but less.	Same as 18 hrs.	Slight increase.
11	"	"	"	"	"	"	"		25	Slight growth.	Same as 18 hrs.	Slight increase.
12	"	"	"	"	"	"	"		30	Slight growth.	Same as 18 hrs.	Slight increase.
13	"	"	"	"	"	"	"		35	No growth noticed.	Same.	Slight growth.
14	"	"	"	"	"	"	"		40	No growth noticed.	Same.	No growth.

TABLE II.

B. COLI COMMUNIS, "COLLECTION."—SURFACE-DROP INOCULATION. NOV. 14, 1896. 4 P. M.

Tube No.	Water.	Meat extract.	NaCl.	Peptone.	Glucose.	Agar.	Gelatin.	NaOH.	HCl.	18 hours. 37° C.	24 hours. 37° C.	48 hours. 37° C.
	cc.	gm.	gm.	gm.	gm.	gm.	gm.	cc.	cc.			
1	1000	5	5	10	10	5	80	25		Some surface growth. No downward extension.		
2	"	"	"	"	"	"	"	20		Surface growth. Slight spreading downward of isolated colonies.		
3	"	"	"	"	"	"	"	15		Surface growth. Some isolated colonies just below surface.		
4	"	"	"	"	"	"	"	10		Same as No. 3. Colonies fewer.		
5	"	"	"	"	"	"	"	5		Same as No. 4. Colonies fewer.		
6	"	"	"	"	"	"	"	neutral.		Surface growth. No colonies.		
7	"	"	"	"	"	"	"		5	Surface growth.		
8	"	"	"	"	"	"	"		10	Surface growth.		
9	"	"	"	"	"	"	"		15	Surface growth.		
10	"	"	"	"	"	"	"		20	Surface growth.		
11	"	"	"	"	"	"	"		25	Surface growth.		
12	"	"	"	"	"	"	"		30	Surface growth confined to original drop.		
13	"	"	"	"	"	"	"		35	Same as No. 12.		
14	"	"	"	"	"	"	"		40	Same as No. 13, but less growth.		
										N. B. Growth less in No. 1 than in No. 2, and decreases from No. 2 as medium becomes more acid.	Practically no change, some slight increase in growth.	Slight increase in growth.

TABLE III.

No.	H_2O.	Meat extract.	NaCl.	Agar.	Gelatin.	NaOH.	HCl.	Peptone.	Glucose.
1	1000 cc.	5 gm.	5 gm.	5 gm.	80 gm.	40 cc.			
2	"	"	"	"	"	35 cc.			
3	"	"	"	"	"	30 cc.			
4	"	"	"	"	"	25 cc.			
5	"	"	"	"	"	20 cc.			
6	"	"	"	"	"	15 cc.			
7	"	"	"	"	"	10 cc.			
8	"	"	"	"	"	5 cc.			
9	"	"	"	"	"	standard = 1.5% acid to phenolphthalein.			
10	"	"	"	"	"		5 cc.		
11	"	"	"	"	"		10 cc.		
12	"	"	"	"	"		15 cc.		
13	"	"	"	"	"		20 cc.		
14	"	"	"	"	"		25 cc.		
15	"	"	"	"	"		30 cc.		
16	"	"	"	"	"		35 cc.		
17	"	"	"	"	"		40 cc.		
18	"	"	"	"	"			10 gm.	
19	"	"	"	"	"			20 gm.	
20	"	"	"	"	"			30 gm.	
21	"	"	"	"	"			40 gm.	
22	"	"	"	"	"				5 gm.
23	"	"	"	"	"				10 gm.
24	"	"	"	"	"				15 gm.
25	"	"	"	"	"				20 gm.
26	"	"	"	"	"				25 gm.
27	"	"	"	"	"				30 gm.
28	"	"	"	"	"				35 gm.
29	"	"	"	"	"				40 gm.

No account was taken of any change in reaction caused by the addition of the various percentages of peptone, although the reaction undoubtedly became more acid to phenolphthalein, nor was any account taken of the change of reaction, if any, caused by the glucose, since it was desired to attribute as directly as possible to these ingredients their effects upon the growth and motility, without masking their rôle by the addition of the alkali necessary to bring them back to the standard reaction. By thus reducing the various media to simple terms it has been possible, in a great degree, to attribute directly to the NaOH, HCl, peptone and glucose their effects on the growth, and to select the most favorable elements for the production either of an optimum combination for growth, or a medium serving for purposes of differentiation.

The media were uniformly inoculated from broth cultures, generally 24 hours old; two series, one inoculated by puncture and one by a single drop of the culture on the surface, have always been under observation.

TABLE IV.

B. TYPHOSUS. "COLLECTION." SURFACE-DROP INOCULATION. NOV. 30, 1896. 4 P. M. THE NUMBERS CORRESPOND TO THOSE OF TABLE III, GIVING THE COMPOSITION OF THE MEDIA.

	No.	18 hours. 37° C.	24 hours. 37° C.	48 hours. 37° C.
Alkaline-acid series.	1	Upper $\frac{9}{10}$ clouded.	Clouded throughout.	Same.
	2	Clouded throughout.	Same as 18 hours.	Same.
	3	Clouded throughout.	Same as 18 hours.	Same.
	4	Clouded throughout.	Same as 18 hours.	Same.
	5	Clouded throughout.	Same as 18 hours.	Same.
	6	Clouded throughout.	Same as 18 hours.	Same.
	7	Clouded throughout.	Same as 18 hours.	Same.
	8	Upper $\frac{7}{8}$ clouded.	Clouded throughout.	Same.
	9	Upper $\frac{6}{7}$ clouded.	Clouded throughout.	Same.
	10	Upper $\frac{1}{2}$ clouded.	Upper $\frac{3}{4}$ clouded.	Same.
	11	Upper $\frac{1}{3}$ misty.	Same as 18 hours.	Same.
	12	Slight downward growth.	Same as 18 hours.	Same.
	13	Slight growth.	Same as 18 hours.	Same.
	14	Slight growth.	Same as 18 hours.	Same.
	15	Very scanty growth.	Same as 18 hours.	Same.
	16	No growth.	No growth.	Slight growth.
	17	No growth.	No growth.	No growth.
Peptone series.	18	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	19	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	20	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	21	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
Glucose series.	22	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	23	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	24	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	25	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	26	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	27	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	28	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.
	29	Upper $\frac{1}{2}$ clouded.	Upper $\frac{2}{3}$ clouded.	Clouded throughout.

TABLE V.

B. COLI COMMUNIS. "COLLECTION." SURFACE-DROP INOCULATION. NOV. 30, 1896. 4 P. M. THE NUMBERS CORRESPOND TO THOSE OF TABLE III, GIVING THE COMPOSITION OF THE MEDIA.

	No.	18 hours. 37° C.	24 hours. 37° C.	48 hours. 37° C.	66 hours. 37° C.
Alkaline-acid series.	1	The NaOH tubes show a slight downward extension of isolated colonies. This increases from tube 1 to tubes 7 and 8 and then steadily decreases as the % of acid becomes larger until tube 17 shows only faintest trace of growth. No clouding occurs.	Increase in growth. No clouding. Same growth proportion between tubes as at 18 hours.	Increase in growth. Otherwise same as 24 hours.	Some clouding in upper ⅓ of Nos. 1, 2, 4, 6. Increase in downward spreading of colonies in all to No. 11.
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				
	13				
	14				
	15				
	16				
	17				
Peptone series.	18	Slight surface growth, but no downward extension.	Increase in growth, same as 18 hours.	Some increase in growth.	Slight increase, only slight downward growth.
	19				
	20				
	21				
Glucose series.	22	Slight surface growth. No downward extension. All tubes appear the same.	Same as 18 hours, but increase in growth.	About same as 24 hours.	Same, no downward spreading.
	23				
	24				
	25				
	26				
	27				
	28				
	29				

Alkaline and acid series.—In the case of the typhoid cultures the alkaline media from the one containing 4 per cent of normal NaOH solution to that of standard reaction have generally given uniform clouding within 24 hours when inoculated by a drop on the surface.

The maximum growth and altogether the quickest clouding occurred in media reacting 2 to 3 per cent alkaline over the standard, *i. e.* 0.5 to 1.5 per cent alkaline to phenolphthalein.

In the media to which normal HCl solution was added the growth was scanty in media containing 1 per cent acid, and none was apparent at 3.5 per cent (see Table IV, p. 686).

For the colon cultures the most favorable media were also those of the alkaline series, and in those they showed a downward growth of limited extent and only in mass or by isolated, well-marked colonies, generally giving rise to no clouding until after about 50 or 60 hours. The growth failed in about the same percentage of acid as the typhoid, *i. e.* 3 per cent to 3.5 per cent (Table V, p. 687).

Peptone series.—The peptone media inoculated with the typhoid bacillus became clouded throughout the upper half within 18 hours, and were completely clouded within 42 hours. The growth appeared somewhat denser than in the alkaline media, but the different percentages had no perceptible effect on the amount of growth or the rate of clouding; as is seen by Table IV, all clouded at the same rate.

With the colon cultures (Table V) the growth was less abundant than in the alkaline media, and less spreading of isolated colonies occurred.

The fact that the peptone media of different percentages permitted the same amount of growth to develop in them is worthy of remark, since the reaction as indicated by phenolphthalein becomes acid in direct proportion to the amount of peptone added, and peptone media permitting growth may be much more acid to phenolphthalein than the media of the acid series in which no growth occurs.

Glucose series.—The glucose media were clouded throughout the upper half within 18 hours by the typhoid growth, and completely clouded within 36 to 42 hours. All the media from that containing the smallest amount, 0.5 per cent of glucose, to that containing 4

per cent of glucose, were clouded at the same rate, and the density of growth was about the same. The rate of clouding was slower than in the medium of standard reaction containing no glucose.

Often the growth of the colon bacillus was limited to the original drop, and no growth extended into the medium. No clouding occurred in any of the series. In some cultures of the less active forms of the colon group, especially among freshly isolated cultures, patches and streaks of growth and bubbles of gas were seen throughout the medium after surface drop inoculations; this I believe is due to some actively motile members of the culture wandering out into the medium and there multiplying into isolated colonies and producing gas. This explanation is applicable to the same appearance occurring after puncture inoculation, although here the gas formed by the growth along the puncture passing up in bubbles also permits the formation of isolated patches and streaks in the wake of these bubbles.

It seems probable that the retardation of the clouding in the typhoid cultures and the prevention of clouding and the limitation of growth in the colon cultures in the glucose media are due to the rapid production of acid from the action of the organisms on the sugar.

Puncture inoculations.—In puncture inoculations of both organisms in the same series of media (Table III) practically the same appearances obtained as in the surface drop inoculation series. The typhoid cultures showed the characteristic clouding of the most favorable alkaline media within 24 hours, or even within 18 hours, and the growth along the line of puncture was hardly more dense than that spreading throughout the medium. In the colon series was found the massing of the growth along the line of puncture, and any outspreading was generally by isolated colonies. In the glucose media was seen a more limited growth along the puncture. Gas was produced in the glucose media, and the most characteristic appearance was given by the isolated masses and streaks of growth occurring in the wake of the ascending gas bubbles. No difference in the amount of growth was shown in the various percentages of glucose; 0.5 per cent seemed to give as much growth and nearly as much gas as the higher percentages.

In the alkaline media, after 50 to 60 hours, a tendency was shown by the colon bacilli to cloud the medium; this was also seen, but to a less extent, in the peptone media, but never did any clouding occur in the glucose media.

One peculiarity of the growth in the peptone media shown by both the typhoid and the colon bacilli, but most markedly by the typhoid, was the early abundant surface growth. This was also at times noticed in the alkaline series, but not to the same extent. The effect of peptone on the growth seems thus to be most marked in the presence of free oxygen.

Reference to the tables, on which are recorded the daily observations, will aid in making clear the above statements (Tables IV and V).

We thus see that in all the media used, with the exception of the acid series, B. typhosus gives a characteristic clouding, and always within a short time, while members of the "colon group" show this tendency to cloud only in the alkaline, and very rarely in the peptone media, and these they cloud only exceptionally and then at a late date. This slight tendency of the less motile type of B. coli communis to cloud has not been noticed in any of the glucose media. Hence the most favorable medium to be used for differentiation purposes, so far as has yet been ascertained, is one containing no peptone and one or more per cent of glucose and of standard reaction, *i. e.* 1.5 per cent acid to phenolphthalein.

Within 12 to 18 hours the growth in such a medium in tube cultures will present a typical picture; and judging from the behavior of the various cultures subjected to trial, this medium may be depended upon to develop one of the most characteristic points in the differential diagnosis of Bacillus typhosus and the less motile varieties of the "colon group."

DIFFERENTIATION OF THE ACTIVELY MOTILE FORMS OF THE COLON GROUP FROM BACILLUS TYPHOSUS.

The actively motile forms of the colon group must now be considered. The series of media inoculated with these organisms gave a very different picture from the series inoculated with the less motile

forms. In range and degree of growth in the various media they corresponded closely with the type already described, but in other respects they simulated more the appearance of the typhoid cultures.

In the alkaline series the media were clouded throughout, quite as rapidly as they were clouded by B. typhosus, and the cultures could be distinguished from those of the typhoid bacillus only by the greater density of the clouding in the colon cultures. The peptone series resembled the alkaline, but with a more abundant growth on or near the surface.

In the glucose series the growth appearances differed from those in the alkaline and the peptone series. Gas was produced in abundance, and its presence alone served to distinguish the organism producing it from B. typhosus. But even after the disappearance of all gas formation, other characteristic differences were present. The media were clouded, with generally a clearer and more fluid stratum at the top, and throughout the media clear spaces occurred, seen best by indirect transmitted light or by holding the tube against the light and slowly passing the finger along the tube between the tube and the light These clearer spaces were oblong and slit-like and seemed to be caused by the gas bubbles in their passage through the medium. The bubbles might often be seen traveling upward along them in a zig-zag course. Streaks and patches of denser growth occurred also throughout the medium and were often seen along the edges of the clear spaces. The surface of the medium just below the superficial clear stratum had an irregularly fissured appearance which was very characteristic. When such a tube was slanted and the medium allowed to harden at the room temperature, the upper thin slanted portion was often perfectly clear and apparently free from organisms. Sometimes the clear stratum was not well marked.

Experiments have been carried on with the hope, which has so far proved vain, of obtaining a medium which would inhibit or limit the motility of the colon bacilli, and at the same time permit of the characteristic clouding of B. typhosus, thereby reducing all the colon growth appearances to one type.

The proportion of the gelatin and agar has been varied in many

ways; for instance, to the litre, combinations of agar 7.5 gm., gelatin 50 gm.; agar 7.5 gm., gelatin 80 gm.; agar 5 gm., gelatin 50 gm.; agar 5 gm., gelatin 100 gm., etc., have been tried, and various sugars and chemicals have been added in different proportions, but these either had no appreciable effect or acted more strongly on B. typhosus than on the colon forms.

It thus happens that the medium which was most advantageous for the differentiation of the less motile species also gave the most typical picture for the motile members of the colon group.

It is of interest to note in connection with the behavior of the actively motile bacilli of the colon group in semi-solid media that descendants of the same colony may display differences in motility. This supports the observations made on the inconstancy of the forms among members of the colon and aerogenes groups. In one case a tube of the medium was inoculated from a colony occurring on a gelatin plate made from feces. This medium gave the appearance typical of the actively motile form of B. coli communis. Afterwards gelatin plate cultures were made from this growth, and colonies varying from each other in appearance were picked out. Media inoculated from some of these colonies were typical of the active form; those from others of the colonies showed these appearances only in sections of the tubes, and others again gave appearances belonging distinctly to the inactive type.

Behavior of other bacteria in the medium.—No systematic attempt has been made to determine the behavior in the medium of organisms other than B. typhosus and the colon bacilli, but sufficient observations have been made to demonstrate that many organisms endowed with motility in no wise simulate the appearances presented by the growth of B. typhosus. The absence of peptone and probably the reaction interfere with the ready development of many of these bacteria. Such well known motile organisms as Proteus vulgaris, Bacillus pyocyaneus, Spirillum cholerae asiaticae and the spirillum of Finkler and Prior, give pictures entirely different from that of the typhoid growth. It is worthy of remark that the cultures of the cholera spirillum and the spirillum of Finkler and Prior at my com-

mand are well differentiated from each other by their growth in this medium.

Uses of the medium.—The practical application of the medium to the work of the isolation of B. typhosus from suspected material will be considered later.

In making cultures from the organs of cases of suspected typhoid fever, almost positive evidence of the absence of B. typhosus is given if the typical clouding does not occur; if it does occur, further tests are of course necessary. If clouding with gas formation takes place, a colon form is probably present, and B. typhosus may or may not be present also.

It was thought when rapid clouding of semi-solid media by the typhoid bacillus was first noticed that an easy and rapid means of separation had been discovered, and that by inoculating fairly thick layers of the medium in flat-bottom flasks or in Petri dishes by a single drop, in the centre, pure cultures of B. typhosus could probably be obtained from the peripheral zone of the clouded area; but the first trials with artificially infected water and feces demonstrated that other organisms present spread quite as rapidly as the typhoid bacillus, and that this method could not be depended upon. These actively motile bacteria were later found to be the active forms of B. coli communis.

DIFFERENTIATION OF THE COLONIES OF BACILLUS TYPHOSUS FROM THE COLONIES OF THE COLON GROUP BY PLATE CULTURE.

In the medium containing 5 gm. of agar, 80 gm. of gelatin, 5 gm. of meat extract, 5 gm. of NaCl, and 10 gm. of glucose to the litre, the colonies of B. typhosus were readily distinguished from the colonies of the non-motile forms of the colon group.

The deep typhoid colonies had no definite outline, and decreased gradually in density from the centre to the periphery; the bacilli spreading through the medium from the colony centre, just as they spread and cloud the medium from the drop or puncture inoculation in the tube. Non-spreading forms rarely occurred. Surface colonies were not observed. This was probably due to the ease with

which the bacilli disseminated themselves throughout the medium, and hence did not become heaped into a surface colony as a less motile organism would.

The non-motile colon forms gave rise to large, roughly spherical, deep colonies, with well defined outlines. These colonies were of a light yellow color when seen with the unaided eye; by transmitted light under low power they were generally of a dark greenish brown. Spreading by means of minute isolated bands of bacilli was seen in some instances. Surface colon colonies were fairly numerous. As seen under low power by transmitted light, they were yellowish brown and granular. They were round or irregular, and generally spread out in a fairly thick layer from the dark colony centre.

When the actively motile colon forms came under observation a more difficult problem presented itself. These forms were found not only to form spreading colonies, simulating the typhoid colonies, but often to cloud rapidly the entire plate and to render it impossible to obtain pure cultures from other colonies in the plate. Many expedients were resorted to in order to limit this spreading, and at the same time to allow characteristic colonies of B. typhosus to develop. The percentages of agar and gelatin were varied, as was also the percentage of glucose, and in addition to these changes the effect of various chemicals was tried, but with the same result as in the tube experiments; the typhoid organisms were more influenced in their development or limited in their motility by these agents than the colon forms.

Finally, after a long series of experiments, a medium was devised, composed of 10 gm. of agar, 25 gm. of gelatin, 5 gm. NaCl, 10 gm. of glucose and 5 gm. of meat extract (Liebig's) to the litre, and of a reaction indicating 2 per cent of normal acid, phenolphthalein being the indicator used. This medium was found to limit almost entirely the spreading of both the typhoid and the colon bacilli, and at the same time to differentiate the colonies of B. typhosus from those of the colon group. As seen under a low power, the deep typhoid colonies are small, generally spherical, with a rough irregular outline, and by transmitted light are of a vitreous greenish or yellow-

ish green color. The most characteristic feature consists of the well defined filamentous outgrowths, ranging from a single thread to a complete fringe around the colony. The young colonies at times are composed solely of threads. The fringing threads generally grow out nearly at right angles to the periphery of the colony, and can be seen by focusing up and down to be directly connected with it. Often there is an angular outgrowth from which the thread arises. The threads may be short, but are often long, and at times even twisted on themselves. A small percentage of the larger deep colonies show no threads. The surface typhoid colonies are small. They usually have a well-defined darker central portion, resembling the deep colony, from which threads may spring, and the part spreading over the surface of the medium is very thin and colorless by transmitted light.

The deep colon colonies are, on the average, much larger than the deep typhoid colonies; they are spherical or of whetstone form, and by transmitted light are darker, more opaque and less refractive than the typhoid colonies. By reflected light, to the unaided eye, they are pale yellow. The surface colon colonies are plentiful, large, round or irregularly spreading, and as seen by transmitted light are brown or brownish yellow. They often show a dark well-defined central spot, and from this spread out in a fairly thick layer over the surface. Their color and size readily differentiate them from the typhoid colonies.

The foregoing descriptions refer to colonies not over 24 hours old and developed at 37° C. Plates should be observed after 16 or 18 hours, for the younger the typhoid colonies the more typical is the thread formation. In old plates the colonies become more dense and approach more closely the colon type, while the older colon colonies may show outgrowths and slight spreading.

If threads occur in the colon colonies, as they in very rare instances do, they are likely to be parallel to the periphery of the colony, and even if at right angles to it, it is generally impossible to determine that they arise directly from the colony. In such a case the large size and the color of the colony, the presence of isolated foci of bacilli,

which are not uncommon around the older colon colonies, and the impossibility of making out the direct joining of the thread and colony, are generally sufficient to prove such a colony not to be typhoid.

In examining plates by the unaided eye the typhoid colonies may often be found without difficulty. They appear as mere greyish points among the much larger and yellowish colon colonies.

The reaction of the medium is most important, and should be as acid as above described. If it is more alkaline the colony appearances of both B. typhosus and colon bacilli change distinctly. In a series of experiments with this medium, to which different amounts of acid and alkali had been added, differences were observed in the shape, size and number of the colonies. In the most favorable medium for growth among both the typhoid and colon forms, the surface colonies became very large and spreading, some of the colon cultures giving rise to very large surface colonies, and the typhoid colonies spreading out into thin transparent films of irregular outline. The deep colonies were increased in size and the typhoid colonies lost their thread formation. As the reaction became acid the surface colonies became more regular, rounder and smaller, and the typhoid surface colonies scarcer. The deep typhoid colonies again became typical and showed thread formation. When the medium became very acid the growth was found again less characteristic and the number of colonies distinctly less.

CONSTANCY OF THE DIFFERENTIATION.

In these experiments, extending over many months, there has never occurred among the numerous organisms examined a culture of B. typhosus that did not present the typical appearance of the large majority of its colonies, nor has there been observed a culture among the various members of the colon group which gave colonies resembling the typical thread-forming typhoid colony. At times colonies are seen which under a low power resemble the typhoid colonies closely, but higher magnification will generally demonstrate the absence of well-defined threads.

No difficulty has been experienced in selecting the typhoid colonies in plates made from mixtures of the various typhoid and colon cultures under observation. The immediate source of the culture does not seem to affect its colony characters. Colonies of B. typhosus from young and old broth cultures, from agar cultures of various ages, and colonies formed by organisms for many days in water, have been found typical, and finally typical colonies develop in plates made from typhoid stools and often in large numbers.

PRACTICAL APPLICATION OF THE COMBINED PLATE AND TUBE METHOD FOR THE ISOLATION AND IDENTIFICATION OF BACILLUS TYPHOSUS.

The method found to be most advantageous in isolating B. typhosus is as follows: It consists in making an inoculation in the tube medium (agar 5 gm., gelatin 80 gm.) from the suspected colony. If the growth in this medium is typical of typhoid, then the usual differential tests for the identification of this organism may be tried, but if the appearance is not typical the organism may without hesitation be eliminated as not typhoid. Up to the present time the writer has not met with an organism other than the typhoid bacillus giving the same combined colony and tube characters in these media as those given by B. typhosus, so that he has come to consider these characters in combination as almost diagnostic.

The tube medium gives us more information than a simple fermentation tube, for we can not only determine that we have an organism which does or does not produce gas, but we can tell something of the motility; and if the appearance given is typical of typhoid, we know that the organism producing it is either B. typhosus or one of the very few organisms which may perhaps give identically the same appearance in this medium. These organisms must be rare, for in all the feces and waters subjected to test none has been found giving an appearance not readily distinguishable from that given by B. typhosus at 37° C. It is thus seen that by this process of elimination the number of organisms to be subjected to ultimate tests is reduced to the minimum.

Since it is necessary to allow the plates to remain in the incubator

at 37° C. for only 16 to 18 hours, and since the growth in the tube medium takes only the same length of time to become characteristic, it follows that by this combined use of the plate and tube media we may obtain within 36 hours almost positive evidence that the organisms tested are or are not typhoid bacilli.

Such a plating medium, which permits of the development of characteristic typhoid colonies at incubator temperatures, presents a distinct advantage in water investigations, since high temperatures kill or prevent the development of the large majority of water organisms.

Further, liquefying organisms cannot spoil the plate, and the plates are only in rare instances overgrown by rapidly spreading forms (hay bacillus group?) and then only after many hours.

Although no suspected water has been subjected to test, still it has been found possible to recover the typhoid organisms without difficulty from artificially infected tap-water. In one experiment of this nature three two-litre flasks filled with tap-water were employed. The first was inoculated with two loops of a 24-hour broth culture of B. typhosus and six loops of a broth culture of the colon bacillus. This flask was well shaken, and one cc. of the water carried over into the second and from this one cc. into the third flask. 0.25 cc. of the water from each of the three flasks was plated and the plates allowed to remain in the incubator at 37° C. for 20 hours. Inoculations were made in the tube medium from typical colonies. These were found in plates from the first and second flasks only, plates from the third flask giving no typical colonies; but of twelve colonies selected from the plates from the first and second flasks, eleven gave the typical clouding in the tube medium, and only one (which was noted at the time of fishing as probably contaminated) gave clouding with gas formation. The eleven gave all the reactions of the typhoid bacillus.

In the stools examined, many colonies have necessarily been tested, in fact all colonies thought to resemble in any particular those of the typhoid bacillus, either in size, color or by the presence of definitely marked threads (though not otherwise characteristic), yet in no case have the organisms proved to be typhoid which did not present a typical colony, *i. e.* in color, size and thread formation.

That other forms presenting threads may occur in water and feces is to be expected, but, as stated above, planting in the tube medium, so far as the writer's experience goes, has always served to differentiate them from B. typhosus.

PREPARATION OF THE MEDIA.

The preparation of the media presents practically no difficulty, and if one uses an autoclave the media may be ready for tubing in an hour and a half. If the agar has to be melted in an ordinary steam sterilizer a longer time is required. The tube medium, consisting of 5 gm. of agar, 80 gm. of gelatin, 5 gm. of beef extract (Liebig's), 5 gm. of sodium chloride and 10 gm. of glucose to the litre, is made by dissolving the agar in 1000 cc. of water to which has been added the beef extract and sodium chloride. When the agar is completely melted the gelatin is added and thoroughly dissolved by a few minutes' boiling. The medium is then titrated to determine its reaction, phenolphthalein being used as the indicator. The requisite amount of normal HCl or NaOH solution is added to bring it to the desired reaction, *i. e.* a reaction indicating 1.5 per cent of normal acid. To clear the medium, add one or two eggs, well beaten in 25 cc. of water, boil for 45 minutes and filter through a thin filter of absorbent cotton. Add the glucose after clearing.

The medium used for plating contains 10 gm. of agar, 25 gm. of gelatin, 5 gm. of beef extract, 5 gm. of sodium chloride, and 10 gm. of glucose. The method of preparation is the same as for the tube medium, care being taken always to add the gelatin after the agar is thoroughly melted, so as not to alter this ingredient by prolonged exposure to high temperature. The glucose is added after clearing. The reaction of this medium is most important, and if, as is usually the case, it is too alkaline, it is rendered of the desired reaction by adding the determined amount of normal solution of HCl. The medium should never contain less than 2 per cent of normal acid.

SUMMARY AND CONCLUSIONS.

Semi-solid culture media, and more especially media rendered semi-solid by temperatures of from 30° to 40° C., seem to have an im-

portant bearing in the differentiation of bacterial species, particularly those presenting various degrees of motility.

In such media not only the effect of differences in consistence on the motility of an organism may be noted, but the effect produced by various chemicals and nutrient ingredients on the growth and motility may be readily observed.

By systematically varying the constituents of such media it has been possible to produce a medium in which the behavior of Bacillus typhosus differentiates it from the various members of the colon group; and also to produce a medium in which the colonies of Bacillus typhosus assume a form which distinguishes them from the colonies of the colon bacilli in plate cultures.

Bacillus typhosus alone of all the organisms investigated during these experiments has displayed both the power of giving rise to thread-forming colonies in the plating medium and that of the uniform clouding of the tube medium, hence these two characters may prove to be of great value in the identification of this organism.

The practical application of the use of these media has led to the ready detection of Bacillus typhosus and its isolation from the stools of patients suffering from typhoid fever.

No suspected water has been subjected to test, but from the investigation of artificially infected tap-water the media here described may be assumed to have an application in the detection of Bacillus typhosus in such waters.

THE BACTERICIDAL ACTION OF LYMPH TAKEN FROM THE THORACIC DUCT OF THE DOG.

By S. J. MELTZER, M. D., and CHARLES NORRIS, M. D.
(From the Physiological and Pathological Departments of the College of Physicians and Surgeons, Columbia University, New York.)

Nine years ago Nuttall* first described the bactericidal action of the blood, suggesting at the same time that the other fluids of the body may possess a similar action. He found later that the aqueous humor, pericardial fluid, and a pleuritic fluid poor in cellular elements also possessed bactericidal power. Prudden† determined that ascitic fluid, fluid taken from a hydrocele, and the liquor amnii of the pig had the same properties.

As far as we have been able to ascertain, no systematic examination of all the body fluids, in regard to this point, has ever been made.

Flexner‡ found that the blood serum of the human placenta was not distinctly germicidal for the Staphylococcus aureus, but was germicidal for the Bacillus typhosus. Caution in drawing conclusions as to the germicidal action of body fluids not hitherto examined is manifestly necessary. A controversy having lately arisen as to the channels of absorption of serous fluids, it seemed to us a matter of importance to determine whether lymph from the thoracic duct possesses bactericidal powers. The possibility was present that an exchange of the bactericidal factors between the blood and lymph—lymph from the interstitial spaces—might occur only through the walls of the blood-vessels. Lymph from the thoracic duct might not therefore possess any action. At the beginning of our work, Professor Prudden called our attention to an article by Max Neisser,§ entitled

* Nuttall, *Zeitschr. f. Hygiene*, iv, 353.

† Prudden, On the Germicidal Action of Blood Serum and other Body Fluids. *Medical Record*, Jan. 25, 1890.

‡ Flexner, *Journal of Experimental Medicine*, i, 576.

§ Neisser, *Zeitschr. f. Hygiene*, xxii, 12.

"Ueber die Durchgängigkeit der Darmwand für Bakterien." In this article Neisser devotes barely ten lines to this question, but nevertheless comes to the conclusion that lymph from the thoracic duct of the dog possesses no germicidal action.

From a scientific point of view it seems important to determine accurately whether lymph, as well as blood, is germicidal, especially as the conclusions of Neisser upon this subject are open to criticism.

The bacteria studied by Neisser with reference to this point were Bacillus prodigiosus, Staphylococcus pyogenes aureus, Bacillus pyocyaneus, and a bacillus resembling Bacillus typhosus. Nissen* furnishes a statement showing the bactericidal power of the blood on Bacillus prodigiosus. Original plate 3000 to 4000, after three hours 2800, after five hours 10,200, after twenty hours innumerable. According to this result blood can hardly be considered to have much germicidal action on this bacillus. In regard to the Staphylococcus aureus, most authors have found that its growth is but little impaired by normal blood, but Flexner† has demonstrated that normal human blood serum may exert decided germicidal action upon this organism. Buchner‡ mentions that the Bacillus pyocyaneus and a bacillus of the typhoid group cultivated from the feces are among the most resistant of the bacteria which he studied, to the action of blood serum. There is no evidence that Neisser's bacillus was identical with that of Buchner, but it certainly would have been well to determine previously the effect of blood serum upon the former. Neisser thus employed organisms over which the blood itself seems to have little or no germicidal power. In testing the germicidal action of lymph and other body fluids, the germicidal action of the blood must be taken as a standard for comparison. When fluids are found to possess no germicidal power over organisms which are likewise resistant to blood, the conclusion is not justifiable that the former fluids possess no bactericidal action. Furthermore, Neisser thus describes his primitive methods of research:§ "Sie wurden theils direct in den Chylus verimpft, theils wurde der Nährboden mit Chylus reichlich vermengt, theils wurden steril gebliebene Versuchsplatten nachträglich mit den Bakterien bestrichen, auf keine Weise aber war eine Behinderung des Bakterienwachsthums nachweisbar." With this imperfect method of experimentation the germicidal action of blood would hardly have been discovered.

* Nissen, *Zeitschr. f. Hygiene*, vi, 488.
† Flexner, op. cit., p. 574.
‡ Buchner, *Centralbl. f. Bakteriol.*, v, 821.
§ Neisser, op. cit., p. 20.

In our investigations the typhoid bacillus was alone tested. This bacillus had been tested by most of the observers working on the germicidal power of the blood, and our main purpose was to establish the presence or absence of any such power in the lymph. The methods employed by us were those followed by Nuttall and others and the method used by Buchner.

It was essential to obtain fluid lymph, as only in this condition can it be readily handled and measured. A sterilized glass cannula bent at right angles is firmly tied in the thoracic duct. The free end of the cannula is then inserted in the neck of a bottle containing glass beads, bottle and beads both having been previously sterilized. After collecting the lymph the bottle is closed with a sterilized glass stopper and vigorously shaken for several minutes. The lymph remains fluid, is poured into test tubes and kept ready for use in an ice chest.

We will not speak of our attempts to obtain fluid lymph by injecting peptone into the circulation or by collecting the lymph in solutions of sodium sulphate or sodium oxalate.

Tying the cannula in the duct and obtaining sterile lymph constitute by no means an easy task, and several of our attempts were unsuccessful. In all our experiments here reported the lymph was proved beyond doubt to be sterile by inoculations in nutrient gelatin and agar watched for three days. We were occasionally obliged to keep the lymph a day or two before testing it. Positive results, however, gain in importance through this circumstance. All the dogs fasted from 30 to 36 hours before being operated upon. The chyle from dogs recently fed is not suitable, as the fine fat droplets interfere in counting colonies on the plates. Artificial respiration was used to stimulate the flow of lymph. In two of the dogs normal salt solution was injected intravenously, whereby a thinner lymph was obtained. In several, small doses of curare were injected. Before and after the flowing of the lymph, specimens of blood were also collected. Unfortunately we were not always able to make parallel tests.

The following results were obtained by the method developed and first employed by Nuttall. 1 cc. of the lymph is pipetted off into a

number of test tubes. 5 cc. of sterilized normal salt solution are inoculated with several loops of a typhoid bouillon culture, thoroughly shaken to insure equal distribution of bacilli throughout the fluid. A fluid gelatin tube under 40° C. is then inoculated with two or three loops of the suspension in salt solution. The gelatin is mixed and poured, this plate serving as the control. All the lymph tubes are then inoculated with the same number of loops of the salt solution and are placed in the thermostat. At the end of the allotted time each lymph tube on removal from the thermostat receives 5 cc. of fluid gelatin (under 40° C.), is then well shaken and poured into a Petri dish. All the plates are kept on a dark shelf at room temperature and the colonies are counted after three days' growth. This method is designated as Nuttall's.

EXPERIMENT F. *Nuttall.*

	Control.	1 hr.	5 hrs.	22 hrs.
Lymph	184	382	20	0

EXPERIMENT G. *Nuttall.*

	Control.	1 hr.	17 hrs.	24 hrs.
Lymph	96	67	18	5

EXPERIMENT K. *Nuttall.*

	Control.	1¼ hrs.	4 hrs.	25 hrs.	48 hrs.	72 hrs.
Lymph	127	15	0	0	0	0

In these experiments the germicidal action of the lymph is apparent. The question presented itself, however, whether the disappearance of the bacilli could not be explained by the lack of suitable nutrient material in the lymph. It might be suggested that the lymph does not kill the bacilli, but that the latter only die from want of suitable nourishment. Although this supposition is extremely improbable, we used two further media for control, one consisting of a normal salt solution containing 1 per cent of bouillon, designated as salt bouillon, of which 0.5 cc. was always used; the other known as lymph bouillon, consisting of 1 cc. of the above salt bouillon, plus 0.5 cc. of lymph.

EXPERIMENT L. *Nuttall.*

	Control.	2 hrs.	5 hrs.	25 hrs.	48 hrs.
Lymph	4130	3339	815	5120	7727
Lymph bouillon	4130	1938	1250	8650	∞
Salt bouillon	4130	5406	25440	∞	∞

EXPERIMENT M. *Nuttall.*

	Control.	2½ hrs.	5½ hrs.	24 hrs.
Lymph	1043	334	264	0
Lymph bouillon	1043	318	215	0
Salt bouillon	1043	11720	9349*	∞
Blood (after flow of lymph)	1043	114	5	140

* Hard to count, colonies spreading on soft gelatin, probably more numerous.

In both experiments growth in the salt bouillon is immediate, the plates soon becoming overcrowded, whereas in the lymph bouillon plates a steady decrease is observed. In Experiment M the 24-hour lymph bouillon and lymph plates are sterile; both series of plates are strikingly similar. In Experiment L, after 25 hours in the thermostat the bacilli begin to grow. This observation corresponds with those already made with the blood, namely, that when the germicidal factors have not been powerful enough to kill all the bacteria, the more resistant surviving forms later begin to grow, and the faster the better the nutritive media presented them. In Experiment L the growth takes place more rapidly in the lymph bouillon than in the plain lymph. The effect of the original number of bacilli on the final result should also be noted. The smaller the number of bacilli exposed to the action of the lymph the sooner are sterile plates obtained. In Experiment L, where the original showed 4130 colonies, a marked decrease alone was observed, the more resistant forms rapidly growing again.

Our next results were obtained mainly by Buchner's method. In each of three sterile test tubes 4 cc. of lymph were introduced. In order to study the effect of heat on the germicidal power of lymph we subjected one of the tubes to half an hour's heat at 56° C. All tubes were inoculated with a typhoid bouillon culture; after shaking, controls were at once made by inoculating melted gelatin tubes with three loops of the lymph. After controls from each of the lymph tubes were made, one tube was kept at room temperature, and the lymph

tube which had been heated for half an hour and the third tube were both placed in the thermostat at 37° C. From all three tubes at regular intervals gelatin tubes were inoculated with the same loop and plates poured. This method is designated as Buchner's. In most of the experiments Nuttall's method was also pursued.

EXPERIMENT N. *Buchner.*

	Control.	1 hr.	3 hrs.	5½ hrs.	24 hrs.	53 hrs.
Lymph 37°	1272	1462	445	3975	45792	∞
Lymph 22°	1481	1580	1106	636	7822	∞
Lymph (heated at 56°) 37°	973	1634	2569	7741	42930	∞
Blood serum,* ante, 37°	1284	775	362	1199	∞	
Blood serum,† post, 37°	2143	2032	1641	∞	∞	

* 4 cc. † 2 cc.

EXPERIMENT N. *Nuttall.*

	Control.	1 hr.	7 hrs.	27 hrs.	48 hrs.
Lymph* 37° C.	886	780	216	0	9471

* Seven days in ice chest before testing.

EXPERIMENT P. *Buchner.*

	Control.	1 hr.	4½ hrs.	26 hrs.
Lymph 37°	1319	550	70	78
Lymph 22°	1648	1399	455	225
Lymph (heated at 56°) 37°	2117	2308	4704	∞
Blood serum, ante, 37°	5083	2026	270	∞
Blood serum, post, 37°	2181	1461	270	∞

EXPERIMENT P. *Nuttall.*

	Control.	1 hr.	2 hrs.	5 hrs.	24 hrs.
Lymph	∞	40068	36061	5787	34344
Lymph bouillon	∞	51643	55014	27475	∞
Blood serum, ante	∞	32626	20606	18899	∞
Blood serum, post	∞	28620	21751	8295	∞

EXPERIMENT R. *Buchner.*

	Control.	1 hr.	4 hrs.	19 hrs.	24 hrs.	72 hrs.
Lymph 37°	906	340	101	196	710	∞
Lymph 22°	1484	969	466	122	89	190
Lymph (56°) 37°	975	954	1643	∞	∞	∞
Blood serum 37°	1696	1229	325	∞	∞	∞
Blood serum 22°	1240	1246	1023	405	217	∞

EXPERIMENT R. *Nuttall.*

	Control.	1 hr.	3 hrs.	5 hrs.	24 hrs.	72 hrs.
Lymph	1310	296	177	140	110	∞
Lymph bouillon	1310	334	140	66	110	6360
Salt bouillon	1310	1900	1060	7268	∞	∞

In Experiment R the lymph was obtained half an hour after the death of the dog. The lymph flowed fifty minutes with the aid of artificial respiration. The blood was obtained out of the central end of the jugular vein.

EXPERIMENT O. *Buchner.*

	Control.	1 hr.	3 hrs.	6 hrs.	26 hrs.
Lymph 37°	1368	1163	3048	16854	∞
Lymph 22°	998	1188	786	1812	∞
Lymph (56°) 37°	1717	1807	2862	∞	∞

EXPERIMENT O. *Nuttall.*

	Control.	1 hr.	3 hrs.	6 hrs.	25 hrs.
Lymph	2353	4293	∞	∞	∞
Lymph bouillon	2353	7504	∞	∞	∞
Salt bouillon	2353	2925	4897	5533	1106

EXPERIMENT O. *Buchner.*

	Control.	1 hr.	3 hrs.	26 hrs.
Blood serum 37°	909	1507	1596	∞
Blood serum 22°	1125	858	975	∞

In Experiment O the blood and lymph remained nineteen days in the ice chest before being tested.

Our results, we believe, justify the conclusion that lymph from the thoracic duct of the dog possesses marked germicidal action on the typhoid bacillus. A noticeable decrease in the number of colonies in the lymph plates is observed, the decrease being the more marked the longer the exposure to the action of the lymph.

The decrease cannot be accounted for simply on account of lack of suitable nutrient material in the lymph, and for the following reasons: The lymph bouillon plates show the same immediate decrease in the number of colonies as do the pure lymph plates, whereas the bouillon salt solution at once shows a marked increase in growth. The addition of lymph produces a direct inhibitory action on the growth of the typhoid bacillus. The germicidal action of lymph is destroyed by too long standing (nineteen days in Experiment O) and by heating for half an hour at 56° C. The lymph after such treatment furnishes a good medium. The action of the lymph is most apparent when the number of bacilli inoculated in the lymph is relatively small. (Compare Experiments F and P: in Experiment F, starting with 184, after twenty-two hours the plate is sterile; in Experiment P (Nuttall) with an

overcrowded original plate a temporary relative diminution alone is observed, growth soon beginning anew.) The quantity of the lymph must also be considered. The greater the quantity of lymph with the same inoculation the more apparent does the action of the lymph become. The bactericidal power of the lymph was rendered much more apparent by Nuttall's method than when Buchner's method was employed. In Buchner's method only two or three loops of lymph are transferred to the gelatin, and this small quantity can hardly continue to exercise any further action, whereas by Nuttall's method the dilution is only one-fifth or one-tenth of gelatin and the action of the lymph in such dilution may very well continue to retard the growth in the Petri dish.

The lymph possesses not merely an inhibitory action on the growth of the typhoid bacillus, but is definitely germicidal. We convinced ourselves of this fact by placing several of the sterile lymph plates in the thermostat. No growth was observed on any of the plates. On the other hand, we have seen lymph plates, which after 3 days' growth at room temperature were sterile, several days later develop a few small colonies, the control plates usually obtaining their full growth in 48 hours. The 5-hour plate in Experiment F furnishes an example. After 53 hours' growth it was sterile, 3 days later 20 colonies appeared. On the whole, a retardation in the appearance of the colonies on the lymph plates as compared with the control plate was always noticeable. The more resistant forms of the bacilli, *i. e.* those not destroyed by the lymph, were nevertheless inhibited in their growth. We believe, however, that the difference between partial and complete inhibition of growth is only a quantitative and not an essential one.

We do not feel justified in forming a positive opinion as to the comparative bactericidal powers of blood serum and lymph, as the number of our successful parallel experiments with lymph and blood serum has not been sufficiently numerous. The experiments here cited, as well as others not published because the lymph or blood serum was not obtained sterile, justify us in believing that the germicidal power of lymph is not much less than that of blood serum.

In one respect a difference between lymph and blood serum was

present in our experiments. The bactericidal action of blood serum diminished and disappeared sooner than did that of lymph. Perhaps blood serum furnishes a better medium for growth, and when the bactericidal property is exhausted the nutritive factors are enabled to gain the upper hand and thus determine a more rapid growth than with lymph.

During the course of our experiments the effect of room temperatures on the bactericidal action of lymph and blood serum was also noticed. Nuttall says "the temperature at which blood specimens after inoculation are kept appears, at least with rabbit's blood, between the limits of 19° and 38° C. to have little effect on the germicidal action." We have, however, obtained a different result. This is evident in a comparison of the development of the germicidal action of lymph and blood in Experiment R, where blood serum or lymph kept at room temperature developed its germicidal power more slowly but retained it longer than blood or lymph kept at 37° C.

NOTES ON THE PHYSIOLOGY OF THE CARDIAC NERVES OF THE OPOSSUM (DIDELPHYS VIRGINIANA).

By REID HUNT and D. W. HARRINGTON.

(From the Physiological Laboratory of Columbia University at the College of Physicians and Surgeons, New York.)

The knowledge of the physiology of the cardiac nerves has been derived from the study of such a very limited number of species that it is desirable to extend the study to other forms. So far as we are aware, no one has investigated the innervation of the heart of a marsupial; hence the following observations upon the opossum may be not without interest.

1. METHODS OF EXPERIMENTING.

As the opossum does not seem to have been used hitherto for physiological experiments, we shall record our observations in some detail.

It may be said that, in general, the opossum is a very favorable subject for physiological experiments; it is of convenient size, the blood-vessels are comparatively large and tough, the blood pressure is moderately high, and anæsthetics are remarkably well borne. In some of these respects the opossum offers marked advantages over the rabbit; on the other hand the large amount of fat which, as is well known, this animal tends to accumulate often makes operations very difficult.

In our experiments the blood pressure and the heart rate were recorded by the mercury manometer in the usual manner.

Anaesthetics.—We have used the following anæsthetics: ether, chloral hydrate (in doses of 1 grm.), morphia sulphate (in doses of .03-.04 grm.) and morphia sulphate and chloral hydrate combined in the proportion of .75 grm. of the latter to .01 grm. of the

former. The morphia and chloral were injected hypodermically. The above mentioned combination of morphia and chloral is probably the best anæsthetic for experiments upon the circulation; animals anæsthetized by it have a good blood pressure; the heart beat is strong and regular, and the vagi are in a condition of moderate tonicity, which seems to be their normal condition.

Normal blood pressure.—The blood pressure was determined only in animals anæsthetized by some of the above drugs; in such animals the arterial pressure, as determined by a cannula in one of the carotid arteries, varied from about 120 to 135 mm. of mercury. Curare affected the blood pressure but little.

Rate of the heart-beat.—The heart rate as determined in anæsthetized animals varied from 144 to 218 beats per minute, being lowest in animals under the influence of morphia alone and highest in etherized animals. When chloral was used, either alone or in combination with morphia, the rate of the beat was between the above mentioned figures. Determinations of the heart rate of the normal animal by means of the stethoscope were not very satisfactory; apparently, however, it was somewhat less than the above—about 120 per minute. Hence the anæsthetic or the operation necessary for the insertion of the cannula had increased the rate of the heart, most probably by diminishing the tonic activity of the vagi.

2. THE VAGUS.

Section of the vagi.—Section of the vagi was followed by results varying in the different experiments according to the degree to which the vagus centres were in activity, and this again varied with the anæsthetic employed. In animals under the influence of chloral or morphia an increase in the heart rate and a rise of blood pressure usually followed section of these nerves; in etherized animals there was little or no effect. Occasionally there was a rise of blood pressure although the heart rate was not affected. Such a rise of blood pressure without any change in the heart rate is not an infrequent occurrence in other animals; the most probable explanation of such cases is that there are fibres in the vagus which on being stimulated

are able to weaken the heart-beat, and that at times they are in a state of tonic activity or have been stimulated in some manner.*

Stimulation of the vagus.—The effects upon the heart of stimulating the peripheral end of the vagus of the opossum are essentially similar to those observed in other mammals; they differ markedly, however, in certain respects.

It seems to us that stimulation of the opossum's vagus is, as a rule, more efficient than is the case with the mammals ordinarily used in the laboratory. For instance, we have never failed to obtain a standstill of the heart by stimulating the vagus with induced currents of moderate intensity (secondary coil of an ordinary Du Bois-Reymond coil 9 to 12 cm. from the primary). As is well known, failure to obtain this result is frequently met with in cats† and occasionally in dogs.

Escape of the heart from stimulation of the vagus.—The heart when brought to a standstill by stimulation of the vagus soon commences to beat again and quickly reaches a "constant level," as described by Hough‡ for the cat and dog. The time required for this escape to occur and the manner of its occurrence and its extent varied greatly. The most usual mode of escape seemed to be that in which the heart, after remaining at a standstill for a short time, suddenly began to beat at a rate which it maintained as long as the stimulation continued. Sometimes this rate was remarkably slow, as is shown in the following experiment:

Feb. 9. Female opossum, etherized. Heart rate 38½ in 10 seconds; blood pressure 102 mm. Hg. Stimulation of the peripheral end of the left vagus with an induced current of moderate intensity caused complete standstill of the heart for 90 seconds. The heart then began to beat at the rate of 5 in 10 seconds, and continued to beat at this rate as long as the stimulation continued (15 minutes), and for some time afterwards. The blood-pressure curve in this experiment is very interesting; with the first three beats of the heart the pressure rose to 64 mm. Hg, or to considerably more than one-half the normal pressure. It fell to 48 mm.

* Cf. Howell, *Journal of Physiology*, xvi (1894), 304.

† This is true of the guinea pig also, as one of us (D. W. H.) has observed.

‡ Hough, *Journal of Physiology*, xviii (1895), 177.

and then rose to 54, at which height it remained during the remainder of the stimulation.

In the above experiment a heart rate of 5 in 10 seconds maintained a blood pressure one-half as great as did a rate of 38½, or nearly eight times as rapid. We shall give later similar instances of the independence of the heart rate and blood pressure.

When the heart was merely slowed by stimulation of the vagus, but not brought to a standstill, a slight escape usually occurred, as was described by Hough for the cat. For example, in one experiment it escaped from 7½ to 10½ beats in 10 seconds, the rate before stimulation having been 26 in 10 seconds. In another case it escaped from 14 to 15 beats in 10 seconds, the vagus having been stimulated when the heart was beating at the rate of 32½ beats in 10 seconds.

After-effect of vagus stimulation.—Perhaps the most marked peculiarity of the effect of stimulating the vagus of the opossum is the fact that the effect upon the heart continues for some time after the stimulation ceases. In the mammals ordinarily used for laboratory purposes and also in the guinea-pig, as one of us (D. W. H.) has observed, the heart after being stopped or slowed by stimulation of the vagus very quickly returns to its normal rate when the stimulation is discontinued; in the dog, in fact, both the heart rate and the blood pressure usually rise considerably above their previous level. In the opossum, however, the return of the heart to the normal rate after stimulation of the vagus is almost always much slower, and we have never observed a secondary acceleration of the heart or a rise of blood pressure. The curve shown in Fig. 1, p. 715, is a good illustration of what usually occurs.

The after-effect varies with the strength of the stimulus, being much more pronounced with a strong than with a weak current. A point of interest is that of two currents of unequal strength, but each of sufficient strength to cause total inhibition, the stronger one causes a longer after-effect. A similar relation between the after-effect upon the heart and the strength of the stimulus is seen when the accelerator nerves are stimulated; of two stimuli, each causing a maximum acceleration, the stronger one causes the longer after-effect.

This after-effect of vagus stimulation is observed when stimuli of very brief duration are employed, so that it can scarcely be attributed to the effect upon the heart of an imperfect supply of blood; besides, as was shown above, the blood pressure usually rises very quickly and sometimes goes above the previous level, although the heart is greatly slowed. Moreover, a local injury to the nerve at the point stimulated, acting as a continuous stimulus, cannot be considered the cause, for cutting off this part of the nerve does not cause the after-effect to disappear. In fact, the long-continued effect upon the heart of vagus stimulation seems to be due to some change in the heart, just as does the long after-effect observed when the accelerators are stimulated.

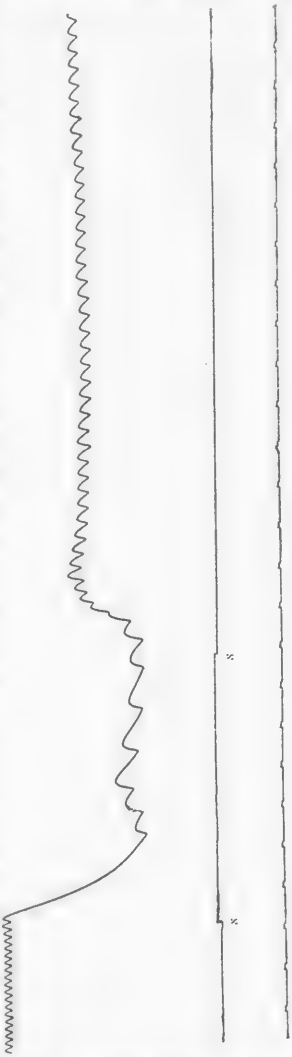


Fig. 1.

Stimulation of the peripheral end of the right vagus (8-8); secondary coil 6 cm. from the primary. The pen used to mark the stimulation also wrote the base line. The time is in intervals of 2 seconds. The heart rate before stimulation was 28 in 10 seconds; in the course of one minute after the stimulation had ceased this rate returned. Curve to be read from left to right.

Effect upon the blood pressure of stimulating the peripheral end of the vagus.—One of the most constantly observed peculiarities of the effect of stimulation of the peripheral end of the vagus is the lack of correspondence between the effect upon the heart rate and the blood pressure. The heart may be greatly slowed and yet the blood pressure remain at the previous level or even rise above it.

Thus in one case the heart rate was slowed from 31 to 23 beats in 10 seconds, or 26 per cent, while the blood pressure rose from 127 to 143 mm. Hg. The same thing is even more strikingly shown in Fig. 2.

In this experiment the heart had been completely inhibited by a strong stimulation of the vagus; on weakening the current slightly the heart began to beat at the rate of 16½ beats in 10 seconds and the blood pressure rose above its previous level, when the heart had been beating at the rate of 37½ in 10 seconds.

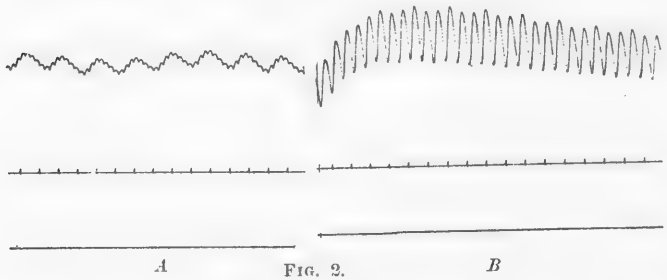


FIG. 2.

Stimulation of the peripheral end of the right vagus. *A* shows the heart rate (37½ beats in 10 seconds) and blood pressure before stimulation. The heart was stopped for several seconds by stimulation of the vagus; the stimulating current was made weaker, the heart began to beat at the rate shown in *B*, and the blood pressure rose and remained at this higher level notwithstanding the slower heart rate. The pleural cavities had been opened and artificial respiration maintained. Time in seconds. Curves to be read from left to right.

That the rise of blood pressure or absence of a fall in these cases is not due to a stimulation of vaso-constrictor nerves, but is due to a direct action upon the heart, is shown by the fact that when atropin is given in sufficient quantity to paralyze the vagus endings, stimulation of the vagus no longer affects the blood pressure. The simplest explanation of this phenomenon appears to us to be that with the slower rate and consequent better filling of the heart the total volume of blood ejected was increased to such an extent that the arterial pressure rose.

These are certainly striking examples of how widely the heart rate

may vary with but slight changes in the blood pressure. A similar phenomenon is frequently observed when the accelerator nerves are stimulated; the heart rate may be greatly accelerated, although the blood pressure is but slightly affected.

3. THE DEPRESSOR NERVE.

We have found a depressor nerve in every opossum but one in which we have looked for it.

Anatomy.—The depressor in the opossum is very similar both anatomically and physiologically to that of the rabbit. It varies somewhat in its origin, but is usually formed by two roots, one from the superior laryngeal nerve and the other from the ganglion of the trunk of the vagus. Sometimes one of these roots seems to be absent; the nerve then arising by a single root.

Stimulation of the depressor.—When the central end of one of the depressor nerves is stimulated with the ordinary faradic current, the blood pressure slowly falls until it reaches a certain level, at which it remains as long as the stimulation continues. After the cessation of stimulation the blood pressure slowly returns to its former height; the return seems to occur more quickly after stimulating with a weak than with a strong current, although the fall of pressure was the same (*i. e.* maximal) in both cases. In all cases a longer time was required for the return than for the fall of pressure.

The maximum effect upon the blood pressure may occur after the stimulation has ceased if this is of brief duration. Thus in one experiment the stimulation continued 20 seconds; before the stimulation the blood pressure was 70 mm.; at the end of the first 10 seconds of stimulation it was 56 mm.; at the end of 20 seconds, 44 mm., and 10 seconds after the cessation of stimulation it was 40 mm., from which height it slowly returned to the normal.

Stimulation of the depressor causes a slight reflex slowing of the heart in case the vagi are intact. If the stimulation is continued for some time the heart rate, as well as the blood pressure, remains at a constant level, differing in this respect from the effect in the rabbit, in which animal the effect upon the heart usually disappears very

soon after the stimulation begins. After the stimulation the heart rate seems to return to the normal sooner than does the blood pressure.

The effect upon the respiration of stimulating the depressor nerve varied in the different experiments; in some, respiration seemed to be not at all affected, while in others it was slowed or completely inhibited for a short time. Sometimes, in the same animal, one depressor affected the respiration while the other did not. The effect upon the blood pressure, however, was always the same, whatever the effect upon the respiration.

Comparison of the effect of stimulating the depressor and the central end of the vagus.—Stimulation of the central end of the vagus was usually followed by a fall of blood pressure. This was true whether the other vagus was cut or intact. The fall of blood pressure in these cases, however, differed as a rule from that following stimulation of the central end of the depressor; in the case of the vagus the blood pressure almost always returned to or rose above its previous level during the stimulation, provided that this was somewhat prolonged, whereas with the depressor the blood pressure remained at a uniform low level throughout. A similar difference has been described by Bayliss* in the effects of stimulating the central end of the vagus and of the depressor in the rabbit.

The respiration was inhibited by stimulation of the central end of the vagus, and it seemed possible that the rise of blood pressure during long-continued stimulation of the vagus described above might be due to stimulation of the vaso-constrictor centre by beginning asphyxia. That this explanation will not hold, however, is shown by the fact that sometimes the blood pressure does not rise after the previous fall during the stimulation of one vagus while it does when the other vagus is stimulated, although the respiration was inhibited in both cases.

Sometimes the effect upon the blood pressure of stimulating the central end of one vagus differed from that resulting from stimulation of the central end of the other and both differed from the typical depressor effect. There is nothing to indicate that in these cases the

* Bayliss, *Journal of Physiology*, xiv (1893), 316.

condition of the vaso-motor centre had changed in the intervals between these stimulations; hence the simplest explanation of these various results is that these different nerves contain fibres having different physiological functions, and the effect upon the blood pressure is determined by the nature and number of the fibres stimulated.*

We may suppose, for example, that some of these nerves contain fibres which cause a fall of blood pressure by inhibiting the vaso-constrictor centre, while others excite the vaso-dilator centre.

4. THE ACCELERATOR NERVES.

On the whole our experiments on the accelerator nerves have not been entirely satisfactory; this has been due, in part, to the fact that these nerves are usually surrounded by fat, which makes their preparation very difficult.

Anatomy.—The anatomy of these nerves in the opossum seems to be very similar to that in the other mammals thus far investigated; apparently, however, there are greater individual variations in the opossum. The first three or four thoracic ganglia of the sympathetic chain are usually united to form one large ganglion, the "stellate ganglion"; occasionally, however, these ganglia are united to form two smaller instead of one large ganglion. The stellate ganglion or the ganglion having the same position as the stellate ganglion is united with the inferior cervical ganglion of the sympathetic by two nerve trunks which form a well marked *annulus of Vieussens*, although at times there is but one nerve connecting the ganglia. The *rami communicantes* passing from the lower cervical and upper thoracic spinal nerves form a plexus from which a variable number of branches pass to the stellate ganglion. Frequently two or three small branches pass from each of the spinal nerves in this region to the plexus. The number of branches passing from the plexus to the stellate ganglion is usually four or five. One or two *rami communicantes* join the sympathetic nerve between the stellate ganglion and the first separate thoracic ganglion. Stimulation of the various *rami communicantes*

* Bayliss, *op. cit.*, p. 317; Howell, *op. cit.*, p. 310; Hunt, *Journal of Physiology*, xviii (1895), 402.

between the last cervical and 3rd dorsal nerves inclusive has caused acceleration of the heart, as has also stimulation of the sympathetic below the entrance of the 3rd *ramus communicans*. From the stellate ganglion the accelerator fibres, as shown by stimulation, pass in part to the inferior cervical ganglion through the nerve or nerves (*annulus of Vieussens*) connecting these ganglia, and in part directly towards the heart from the stellate ganglion or in small branches given off from the annulus. In a few animals a comparatively large nerve, evidently corresponding to the "*nervus accelerans*" of the cat, passed from the stellate ganglion directly towards the heart; stimulation of this nerve caused marked acceleration.

Stimulation of the accelerator nerves.—In most respects the effect of stimulating these nerves is the same as in other mammals—an increase in the heart rate, with or without a rise of blood pressure. Perhaps the most interesting point which we have observed is the tendency of the ventricle to beat irregularly when they are stimulated. Very frequently the first effect of the stimulation was to cause a slowing of the rate of the ventricle; this was followed, either during or after the stimulation, by a more rapid but often irregular rate. Judging from what we have seen with great ease in the experiment upon the calf described in the following paper and from less satisfactory observations upon the opossum's heart, we are confident that the slowing and irregularity of the ventricle were due to the ventricle's failing to respond to all the beats of the auricles. Sometimes the ventricular beat followed each auricular beat and at other times only every second or third beat. This irregularity of the ventricle was most marked when strong stimuli were applied to the accelerators; still it occurred also when weak stimuli were applied so that the total effect upon the heart was slight. As a rule after this slow or irregular rate had continued for a variable length of time, it was suddenly replaced by a uniform rapid rate. In other cases the first effect of stimulation was a slight acceleration which soon gave way to the slow or irregular rate as the stimulation continued; after the stimulation the rapid regular rate appeared again. In some cases the acceleration was very considerable, *e. g.* in one experiment there was an increase from 23 to 41½ beats in ten seconds, or an acceleration

of about 80 per cent resulting from the stimulation of the above-mentioned branch of the stellate ganglion ("*nervus accelerans*").

The effect upon blood pressure of stimulating these nerves was variable. As a rule the blood pressure was but little affected even when the acceleration was considerable, resembling in this respect the result so frequently obtained in the dog and cat. Thus in the experiment mentioned above in which there was an acceleration of 80 per cent, there was no change in the blood pressure.

In other respects the effect of stimulating the accelerator nerves in the opossum is similar to that observed in other mammals. Thus a moderately strong stimulus is required for an effect to be produced, and the difference between the strength of a minimal and a maximal stimulus is not great. The latent period is long and there is a long after-effect. Sometimes during the after-effect there is a sudden slowing of the heart rate, although it remains above the normal; from this new rate there is a gradual return to the normal. For example, in one case the rate before stimulation was 23 beats in 10 seconds, soon after stimulation it was 38; it then suddenly changed to 27, from which rate it slowly dropped to 24. We know of no explanation of this peculiarity.

The following figures from one of our experiments are interesting as bearing upon the relation of the inhibiting to the accelerator nerves: First the accelerator nerves were stimulated alone and the heart rate increased from 32 to $38\frac{1}{2}$ beats in 10 seconds (*i. e.* there was an acceleration of 20 per cent). The vagus was now stimulated for some time; the heart rate fell from 33 to $13\frac{1}{2}$ in 10 seconds; during the stimulation of the vagus the accelerators were stimulated again with the same strength of current as before; the rate increased to 20 (*i. e.* there was an acceleration of 48.1 per cent, or a greater acceleration relatively than when the accelerators were stimulated alone).

Obviously the effect in this case of stimulating the two nerves simultaneously was the resultant of the effects of stimulating the nerves separately. This result agrees entirely with what one of us* has found to be the case in experiments upon dogs and cats.

* Hunt, *Journal of Experimental Medicine*, ii (1897), 151.

NOTE ON THE PHYSIOLOGY OF THE CARDIAC NERVES OF THE CALF.

By REID HUNT and D. W. HARRINGTON.

(From the Physiological Laboratory of Columbia University at the College of Physicians and Surgeons, New York.)

We were able to experiment upon but one calf, and had we any expectation of continuing this investigation in the near future we should not publish the results of this experiment at present. Our results, however, seem to us so satisfactory and conclusive that we feel justified in describing them. The heart of the calf is so large and the rate of beat so slow that every change can be followed by the eye with great ease, and the effects of stimulating various nerves can be determined more satisfactorily in some respects than when recording apparatus is applied to the smaller and more rapid hearts of the animals ordinarily used in the laboratory.

The calf upon which we experimented had been used by Dr. Curtis in a lecture demonstration and had been under the influence of ether for some time, but the heart was beating vigorously, and the blood pressure, which was recorded by the mercury manometer in the usual manner, was moderately high (100 mm. of mercury).

Stimulation of the vagus.—Stimulation of the peripheral end of the vagus with currents of moderate intensity caused slowing of both auricle and ventricle. In a number of cases, however, the ventricle was slowed very decidedly, while the rate of the auricle was but slightly affected. This result agrees with that described by Franck* for the dog, but differs from the results obtained by most experimenters for this animal. McWilliam,† Bayliss and Starling,‡ and Roy and Adami,§ for example, speak of the auricles in the dog as being

* Franck, *Archives de Physiologie*, 5. s., ii (1890), 403.

† McWilliam, *Journal of Physiology*, ix (1888), 345.

‡ Bayliss and Starling, *ib.* xiii (1892), 407.

§ Roy and Adami, *Phil. Trans.*, clxxxiii (B), (1892), 199.

more easily slowed by stimulation of the vagus than are the ventricles. In none of the several stimulations of the vagus in the calf did we observe a slowing of the auricles without a slowing of the ventricles also.

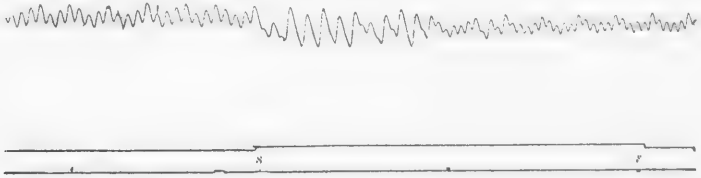
It is interesting to note that sometimes there was an after-effect of the stimulation of the vagus in which the effect upon the ventricle was more marked than that upon the auricles. Thus in one case, after a long-continued stimulation of the vagus, during which auricles and ventricles were both slowed but continued to beat together, the ventricles did not return to their normal rate, while the auricles did return, and it was observed that the ventricles were following at times every auricular beat, and at other times only every second beat. While this slow rate of the ventricles continued, the accelerators were stimulated and the ventricles began at once to follow all the auricular beats. The rate of the auricles, however, was but slightly increased. After the cessation of the stimulation of the accelerators the slow irregular beat of the ventricles returned.

Apparently these results and similar ones obtained in the experiments upon the opossum can be explained satisfactorily by supposing the conductivity of the muscle fibres connecting the auricle and ventricle to have been altered by the stimulation of the nerves.* After the prolonged stimulation of the vagus, the conductivity of these muscle fibres seemed to be depressed for some time; stimulation of the accelerators apparently caused a temporary increase of their irritability, and as a consequence the ventricle followed every auricular beat. So, also, the decreased conductivity of these fibres resulting from the action of the vagus may explain why stimulation of these nerves at times caused a slowing of the ventricles while the auricles were not much affected. Possibly this factor—the changes in the conductivity of the muscle fibres between the auricles and ventricles—has not received sufficient attention from some of the physiologists who have discussed the relative effects of the stimulation of the vagus and accelerator nerves upon the different parts of the heart.

* *Cf.* Gaskell, *Journal of Physiology*, iv (1883), 100; McWilliam, *op. cit.* 367; Bayliss and Starling, *op. cit.* 412.

Stimulation of the peripheral end of the vagus with a very weak current caused in one or two cases a slight acceleration of the heart, as is often observed in the dog.

Accelerator nerves.—We stimulated two nerves passing from the spinal nerves to the stellate ganglion—the *ramus communicans* from the second spinal nerve and a nerve accompanying the vertebral artery (the "*nervus vertebralis*"). Stimulation of both of these nerves caused acceleration of the heart; the results differed, however, in a very interesting manner according as one or the other nerve was stimulated. The immediate effect of stimulating the vertebral nerve was to cause an acceleration of the auricles, but a slowing of the ventricles due to failure of the latter to follow all of the beats of the former. Frequently, if the stimulation was continued for some time, the ventricles began to follow all the auricular beats. We have in this case an illustration of how stimulation of the accelerator nerves can cause an actual slowing of the ventricles—a possibility recently pointed out by Engelmann.* This primary slowing was always obtained whatever the strength of the stimulus used; the acceleration of the auricles and the slowing of the ventricles was most marked, however, with strong stimuli.



Stimulation (*s-s*) of the vertebral nerve; the secondary coil was 12 cm. from the primary. In the early part of the stimulation the ventricles failed to follow all the auricular beats, and the result was a slight slowing of the pulse; later there was an acceleration, the ventricles now beating at the same rate as the auricles. Time in intervals of 10 seconds. Curve to be read from left to right.

During the stimulation, the result of which is shown in the accompanying figure, the secondary coil of the induction apparatus was 12 cm. from the primary coil; the rate of the auricles and ventricles

* Engelmann, Pflüger's *Archiv*, lxv (1896), 161.

before stimulation was 20 beats in 10 seconds. In the first 10 seconds during stimulation the auricle gave 24 beats, the ventricle but 19, as it failed to follow all the auricular beats; in the second period of 10 seconds both auricle and ventricle gave 26 beats and the latter followed the former throughout. In a second stimulation the stimulus was stronger, the coil being at 10 cm., before stimulation the auricle and ventricle were beating at the rate of 19½ in 10 seconds; during stimulation the auricles beat at the rate of 26 in 10 seconds, while the ventricles, following only every second auricular beat, had a rate of 13 in 10 seconds. Later the rate of the ventricle became more rapid, following all the auricular beats.

The effect upon the heart of stimulating the 2nd *ramus communicans* differed from the above in that both auricle and ventricle were uniformly accelerated. The following figures give the result of stimulating the 2nd *ramus communicans* with the coil at 10 cm. (the same strength of current as was used in the second of the above stimulations of the vertebral). The rate of both auricle and ventricle before stimulation was 20 in 10 seconds; during the first 10 seconds the rate increased to 23½, during the second 10 seconds it was 28, during the third, 28½, and during the first 10 seconds after stimulation it was 29½. Throughout the stimulation each auricular beat was followed by a ventricular beat, and this occurred also when stimuli of various intensities were used.

The above facts show that in this individual at least the accelerator fibres passing through the vertebral nerve were different from those in the 2nd *ramus communicans*; stimulation of each nerve caused acceleration of the auricle, but the fibres of the 2nd *ramus communicans* evidently had more effect upon the ventricle than did those of the vertebral. This difference may be explained by supposing that the *ramus communicans* contained more fibres passing to the ventricle than did the vertebral nerve, or, and this is perhaps more probable, by supposing that it contained more fibres, stimulation of which caused the conductivity of the muscle fibres connecting the auricles and ventricles to be increased. In any case, it is certain that the accelerator fibres in the two nerves differed in either their ana-

tomical or physiological relations to the heart. Bayliss and Starling* observed a similar separation of the functions of the accelerator fibres in the dog and ascribed the result to the earlier fatigue of one set of fibres; the element of fatigue can probably be excluded from our experiment upon the calf, for both nerves had received the same treatment.

We also tried the effect of stimulating the inhibitory and accelerator nerves simultaneously. The peripheral end of one vagus was stimulated with an induced current produced by the slow interruption of the primary current with the "Schlagwähler"; both auricles and ventricles were slowed. While the stimulation of the vagus was continued the accelerators were stimulated; the rate of both auricles and ventricles was accelerated.

Thus the antagonism of the inhibitory and accelerator nerves, already found to hold in the case of the ventricles of other animals, is found to occur in the calf also, and holds for the auricles as well as for the ventricles.†

* Bayliss and Starling, *op. cit.* p. 416.

† Since the completion of my paper upon the relation of the inhibitory to the accelerator nerves of the heart (*Journal of Experimental Medicine*, ii (1897), 151), I have repeatedly observed directly the effect upon the auricle and ventricle of the simultaneous stimulation of these nerves in the dog. With the strength of current which I used, a ventricular always followed an auricular beat, and in all cases the effect upon the rate of the auricle as well as upon that of the ventricle, was approximately the algebraic sum of the effects produced by stimulating the nerves separately. An "independent ventricular rhythm" which might complicate the results was never caused by the stimulation of the vagus in my experiments.—R. H.

ERRATUM.

Vol. II, page 391. The last word of the first paragraph should be "wall," instead of "septum."

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COLLABORATORS

A. C. ABBOTT, M. D., Professor of Hygiene, University of Pennsylvania.

L. F. BARKER, M. D., Associate Professor of Anatomy, Johns Hopkins University; Assistant Pathologist, Johns Hopkins Hospital.

JOHN S. BILLINGS, M. D., U. S. Army (retired), New York City.

HERMANN M. BIGGS, M. D., Professor of Materia Medica, Therapeutics, and Clinical Medicine, Bellevue Hospital Medical College; Pathologist and Director of the Bacteriological Laboratories of the Health Department of New York City.

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H. C. ERNST, M. D., Professor of Bacteriology, Harvard University.

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GEORGE W. FULLER, B. S., Chief Chemist and Bacteriologist to the Louisville Water Company; late Biologist in charge of the Lawrence Experiment Station, State Board of Health of Massachusetts.

J. GUITERAS, M. D., Professor of General Pathology and Morbid Anatomy, University of Pennsylvania.

WINFIELD S. HALL, Ph. D., M. D., Professor of Physiology, Northwestern University Medical School, Chicago.

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LUDVIG HEKTOEN, M. D., Professor of Morbid Anatomy, Rush Medical College, Chicago.

CHRISTIAN A. HERTER, M. D., New York City.

EUGENE HODENPYL, M. D., Assistant in Pathology, Columbia University.

C. F. HODGE, Ph. D., Assistant Professor of Physiology and Neurology, Clark University.

W. T. HOWARD, Jr., M. D., Professor of Pathology, Western Reserve University.

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WILLIAM H. PARK, M. D., Assistant Director of Hospital Bacteriological Laboratory of the Health Department of New York City.

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F. F. WESBROOK, M. D., Professor of Pathology and Bacteriology, University of Minnesota.

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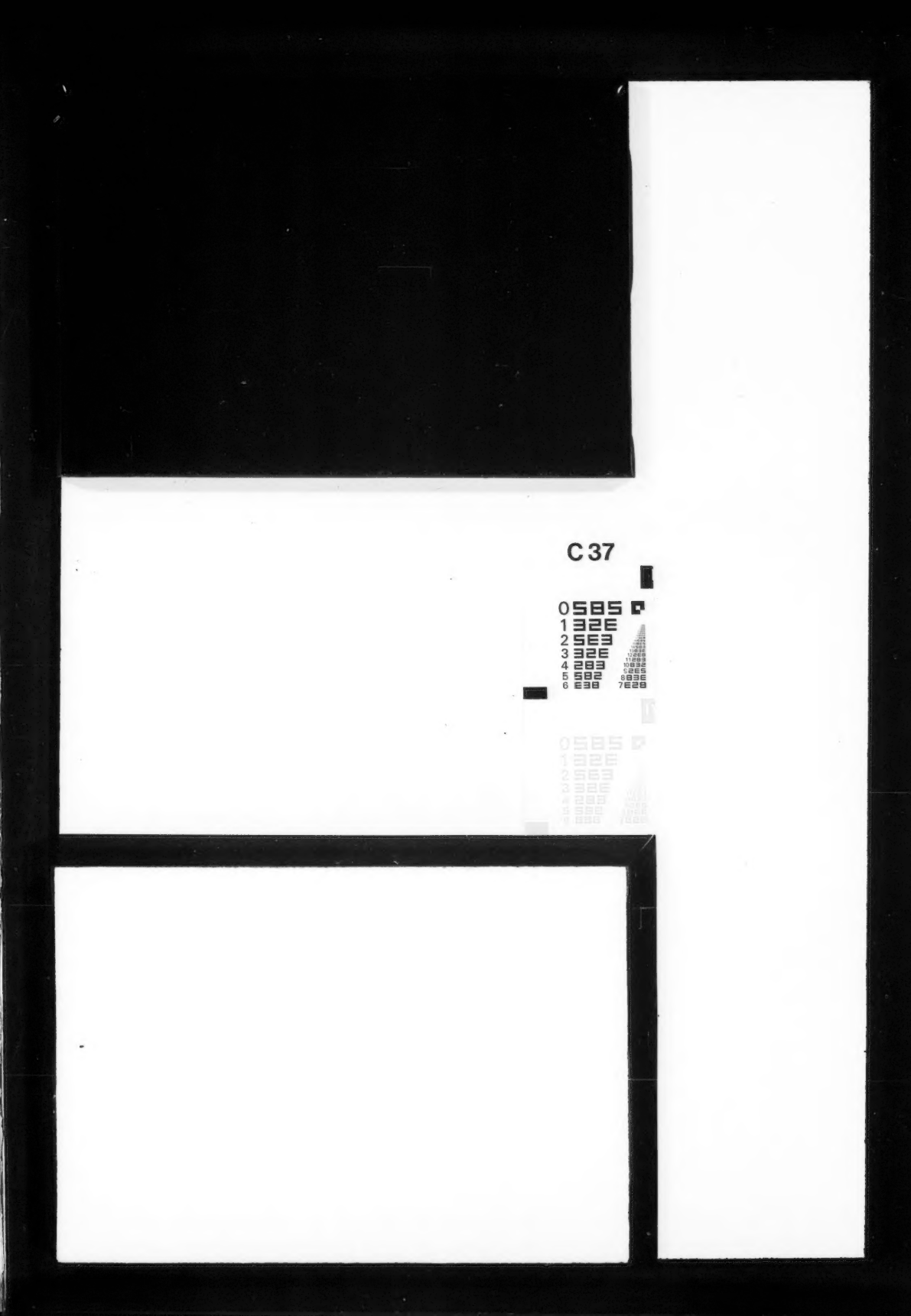
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